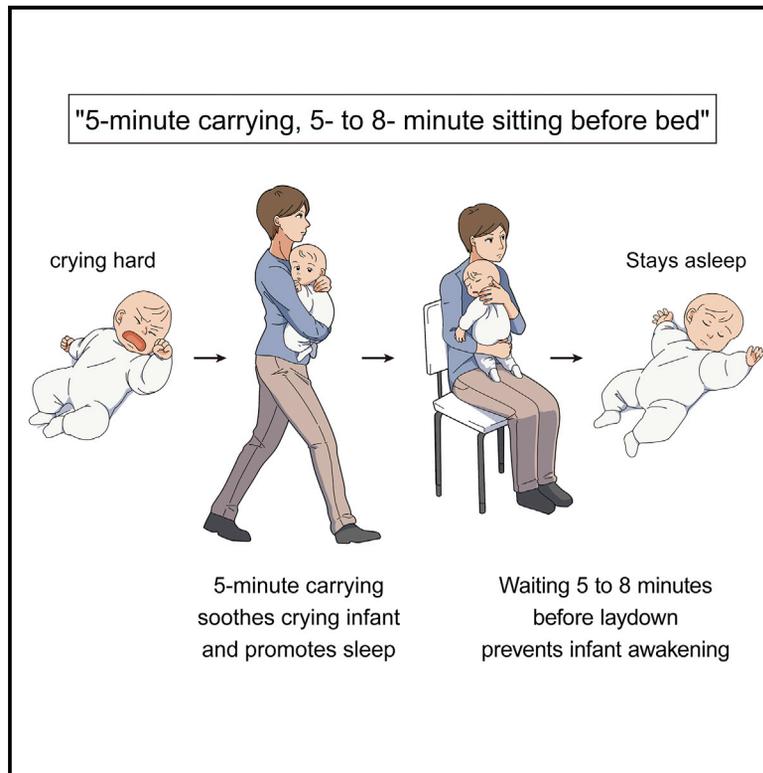


Current Biology

A method to soothe and promote sleep in crying infants utilizing the transport response

Graphical abstract



Authors

Nami Ohmura, Lana Okuma, Anna Truzzi, ..., Masaki Shimizu, Gianluca Esposito, Kumi O. Kuroda

Correspondence

gianluca.esposito@unitn.it (G.E.), kumi.kuroda@a.riken.jp (K.O.K.)

In brief

Ohmura et al. investigate the effects of maternal holding and carrying on infant behavioral states (crying, alert, asleep) and identify that transport soothes crying infants and potentially promotes sleep. This study proposes “5-min carrying, 5- to 8-min sitting for bed” as an on-site behavioral intervention for infant crying and sleep difficulties.

Highlights

- Infant cry is attenuated by transport, but not by motionless holding
- 5-min transport promotes sleep for crying infants even in the daytime
- Laydown of sleeping infants into a cot either interrupts or deepens infants' sleep
- Laydown at 5 to 8 min after the sleep onset tends to prevent infant awakening

Report

A method to soothe and promote sleep in crying infants utilizing the transport response

Nami Ohmura,^{1,8} Lana Okuma,^{1,2} Anna Truzzi,^{1,3,4} Kazutaka Shinozuka,¹ Atsuko Saito,^{1,5} Susumu Yokota,⁶ Andrea Bizzego,³ Eri Miyazawa,¹ Masaki Shimizu,⁷ Gianluca Esposito,^{1,3,*} and Kumi O. Kuroda^{1,9,10,*}

¹Laboratory for Affiliative Social Behavior, RIKEN Center for Brain Science, Saitama 351-0198, Japan

²Laboratory for Human Cognition and Learning, RIKEN Center for Brain Science, Saitama 351-0198, Japan

³Department of Psychology and Cognitive Science, University of Trento, Rovereto, Italy

⁴Trinity College Institute of Neuroscience, School of Psychology, Trinity College Dublin, Dublin, Ireland

⁵Department of Psychology, Sophia University, Tokyo 102-8554, Japan

⁶Department of Clinical Laboratory, Saitama Prefectural Cardiovascular and Respiratory Center, Saitama 360-0197, Japan

⁷Division of Neonatology, Saitama Prefectural Children's Medical Center, Saitama 330-8777, Japan

⁸Twitter: @NamiOhmura

⁹Twitter: @kumi_kuroda

¹⁰Lead contact

*Correspondence: gianluca.esposito@unitn.it (G.E.), kumi.kuroda@a.riken.jp (K.O.K.)

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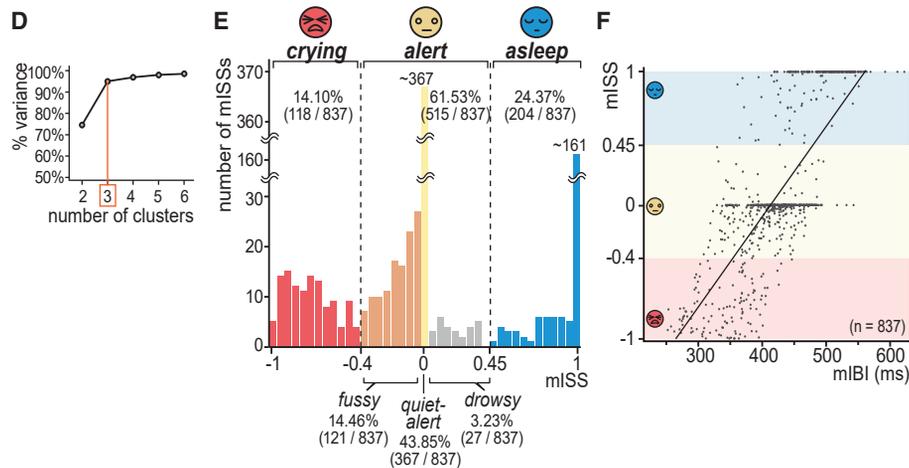
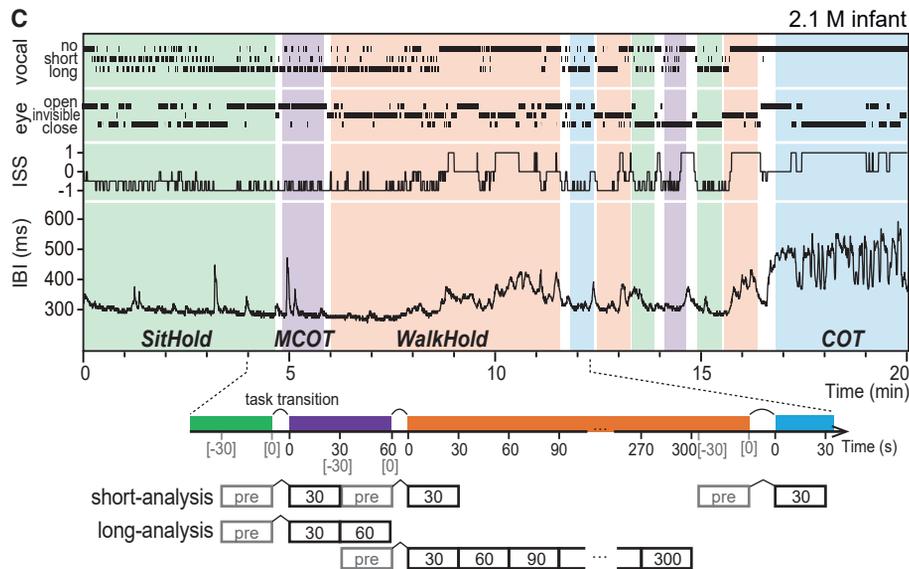
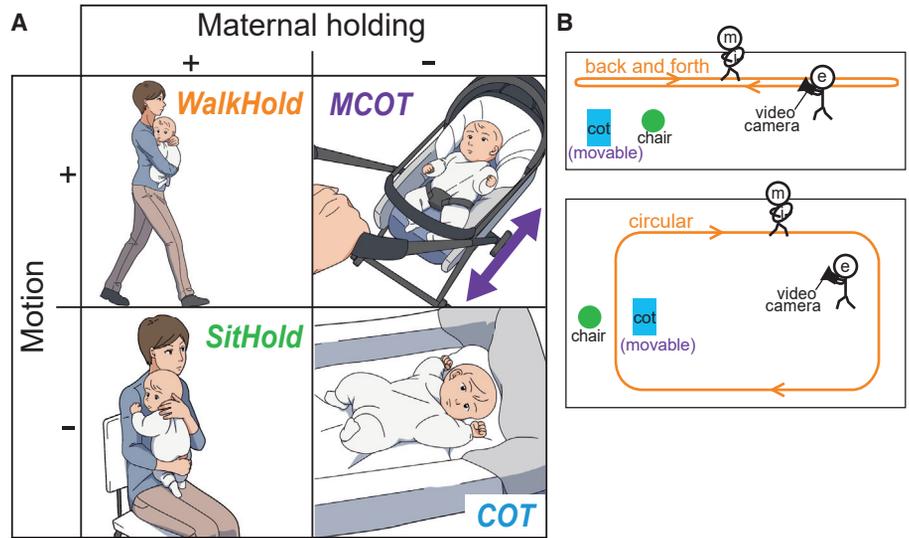
SUMMARY

Approximately 20%–30% of infants cry excessively and exhibit sleep difficulties for no apparent reason, causing parental stress and even triggering impulsive child maltreatment in a small number of cases.^{1–8} While several sleep training methods or parental education programs may provide long-term improvement of infant cry and sleep problems, there is yet to be a conclusive recommendation for on-site behavioral interventions.^{9–13} Previously we have reported that brief carrying of infants transiently reduces infant cry via the transport response, a coordinated set of vagal activation and behavioral calming conserved in altricial mammals.^{14–18} In this study, we disentangled complex infant responses to maternal holding and transport by combining subsecond-scale, event-locked physiological analyses with dynamic mother-infant interactions. Infant cry was attenuated either by maternal carrying or by reciprocal motion provided by a moving cot, but not by maternal holding. Five-minute carrying promoted sleep for crying infants even in the daytime when these infants were usually awake, but not for non-crying infants. Maternal laydown of sleeping infants into a cot exerted bimodal effects, either interrupting or deepening the infants' sleep. During laydown, sleeping infants were alerted most consistently by the initiation of maternal detachment, then calmed after the completion of maternal detachment in a successful laydown. Finally, the sleep outcome after laydown was associated with the sleep duration before the laydown onset. These data propose a “5-min carrying, 5- to 8- min sitting” scheme for attending to infant cry and sleep difficulties, which should be further substantiated in future studies.

RESULTS AND DISCUSSION

This study employed four conditions (Figure 1A): (1) WalkHold, the mother held the infant and walked (Figure 1B); (2) SitHold, the mother held the infant and sat; (3) COT, the infant was laid in a cot; and (4) MCOT, the infant was placed in a mobile crib or stroller and was moved in a reciprocal motion manually. The MCOT was performed only when these devices were made available by the participants. This experimental design segregates the effects of maternal holding and motion (Figure 1A), the two sensory components involved in the induction of the transport response.¹⁴ Each task lasted either 30 s (short condition) or 5 min (long condition) and was alternated randomly in one session (Figure 1C). The mothers were informed that they could skip a condition when they felt tired or uncomfortable letting their infant cry.

For physiological and behavioral measurements, we used a Holter electrocardiogram (ECG) and a hand-held video camera, respectively, not to disturb the naturalistic interactions of the dyads (STAR Methods). Inter-beat intervals (IBIs) were calculated from the infant ECG as the time intervals between consecutive heartbeats¹⁹ and were averaged for a 30-s period to yield the mean IBI (mIBI). For the infants' behavioral state, we analyzed the presence of infant negative vocalization and eye opening/closure at each heartbeat (one to four points/s) (Figure 1C, top two rows). Then, based on the previous qualitative categorization of infant state,²⁰ an arbitrary infant status score (ISS) was created and operationally assigned to each heartbeat (Figure 1C, third row) to code behavioral sleep (ISS = 1), quiet alert (ISS = 0), and hard crying (ISS = -1) states. An unsupervised cluster analysis of the 30-s mean ISSs (mISSs) yielded cluster borders at -0.4 and +0.45, which were then used as



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thresholds for infant state categorization at each time bin (Figures 1D and 1E; Video S1). The resultant mISS correlated robustly with mIBI ($\rho = 0.75$, $p < 0.001$) (Figure 1F).

We examined the effects of WalkHold, SitHold, COT, and MCOT on infant ISS and IBI separately for the infants who were crying, alert, and asleep at the end of the preceding condition (“pre” in Figure 2), partly based on our previous study.¹⁴ For the crying-start infants, short WalkHold and MCOT decreased cry (Figures 2A and S1) and increased the mISS and mIBI (Figures 2B and 2C). SitHold did not alter the ratio of crying infants or mISS and decreased mIBI only (Figures 2A–2C). The mIBI decrease by SitHold was pronounced when the preceding condition was COT (Figures 2C and S1A–S1C), which might reflect the autonomic cardiovascular adaptation to the postural change from supine to upright.²¹ Conversely, transitions to COT did not affect mISS but slightly increased mIBI (Figures 2B and 2C).

For the alert-start (awake and not crying) infants, WalkHold or MCOT did not affect mISS or mIBI (Figures 2D–2F). However, WalkHold increased the mIBI when the preceding condition was SitHold (Figures S1D–S1F), consistent with our previous finding.¹⁴ Unexpectedly, SitHold decreased both mISS and mIBI of alert-start infants (Figures 2E, 2F, S1E, and S1F). COT decreased mISS, and marginally mIBI (Figure 2F, $p = 0.059$; Figures S1D–S1F). In contrast, asleep-start infants increased their mIBI significantly by COT, but not by WalkHold or MCOT (Figures 2G–2I), suggesting that the sleeping infants could be further calmed by cessation of sensory stimuli caused by motion (Figures 4B–4D).

We then performed linear mixed-model (LMM) analyses to evaluate the fixed effects of motion, holding, maternal and infant age, infant sex, the location of the experiment, and the infant state at the beginning of the session. The infant ID was set as a random effect. The presence of motion was most robustly associated with both the mISS and mIBI increase in crying-start infants (mISS, $t = 6.28$, $df = 54.26$, $p < 0.001$; mIBI, $t = 6.57$, $df = 54.12$, $p < 0.001$). The presence of holding was mildly associated with mIBI decrease ($t = -2.14$, $df = 54.17$, $p = 0.037$), but not with mISS ($t = 0.49$, $df = 46.34$, $p = 0.629$), at least partly due to the postural changes as mentioned above. The LMM analysis also revealed a weak positive correlation between infant age and mIBI ($t = 2.14$, $df = 19.02$, $p = 0.045$). Other explanatory variables were not associated with mISS or mIBI changes.

For alert-start infants, the LMM analysis revealed an effect of motion on both mISS ($t = 2.98$, $df = 114.45$, $p = 0.004$) and mIBI ($t = 4.94$, $df = 123.39$, $p < 0.001$), and a weak effect of holding on mISS ($t = 2.08$, $df = 119.25$, $p = 0.040$), suggesting that

maternal holding was mildly effective in calming alert infants. Other explanatory variables were not reliably associated with either mISS or mIBI changes (STAR Methods). These data suggested that the rhythmic motion associated with WalkHold and MCOT had robust calming effects on both crying and alert infants, possibly via proprioception or the vestibular system.^{14,22,23}

Long WalkHold up to 5 min on crying-start infants (Figures 3A and 3B; Video S2) decreased the ratio of crying infants and increased the mISS beyond the initial effects described above. After a 5-min WalkHold, 5 of 11 infants (45.5%) were asleep and no infants were crying by the end of the task, and two additional infants (18.2%) fell asleep within 1 min of the succeeding SitHold († and ‡ in Figures 2E and S2B; see Video S3 for †), which itself did not seem to promote sleep. Consistently, the difference from the initial mIBI (Δ mIBI) increased gradually until 210 s (Figure 3C). These data indicate that crying infants are calmed and are inclined to sleep by a 5-min WalkHold, even in the daytime when the infants are normally awake.

Contrary to our expectation, alert-start infants were not promoted to sleep by a long WalkHold, nor did they show significant changes in mISS or mIBI (Figures 3D–3F). As a result, crying-start infants overtook alert-start infants in both mISS and mIBI at the end of the long WalkHold (Figure S2). Crying infants might be more tired than non-crying infants and therefore more prone to sleep, triggered by reflexive vagal activation of the transport response.¹⁴

A long SitHold did not calm crying infants (Figure S2A), leading to frequent termination of the task. COT was even more difficult to continue with hard-crying infants, as picking up an infant is reported to be the most culturally common maternal response to infant cry,²⁴ and also because the hard-crying older infants often moved around by rolling and kicking. In one case that lasted for 180 s, the cry remained till the end without signs of waning (Figure S2A). The sample size of long MCOT was very small, but long MCOT results seemed consistent with the results from short MCOT (Figure S2).

For alert-start infants, SitHold and COT acted similarly; all the infants who fell asleep during these tasks (†, ‡, and § in long SitHold and # in long COT in Figure S2B) were those who had experienced a long WalkHold as the preceding condition. Also, for all the infants who started crying hard and their mISS reached below -0.6 , their mISS did not return to values above -0.4 . These observations suggested that SitHold could not effectively resolve infant cry, contradicting a general assumption that maternal holding reduces infant distress.²⁵ The previous studies

Figure 1. Experimental design

(A) The four maternal tasks: WalkHold, SitHold, COT, and MCOT. See text for explanation.

(B) A typical room layout. Mothers walked back and forth (top) or circularly (bottom) as indicated by the orange line. m, mother; i, infant; e, experimenter.

(C) An example of the experiment with a 2-month-old infant and its mother. Top panel: time courses of infant negative vocalization (vocal), eye state, infant status score (ISS), and infant IBI with maternal task transition. Each maternal task was divided by shaded color: orange, WalkHold; green, SitHold; light blue, COT; purple, MCOT. Bottom panel: schematic of 30-s binning for the following analysis. The conditions were divided into 30-s bins and were analyzed in pairs with the “pre” that referred to the last 30-s bin of the preceding condition.

(D) The elbow analysis. The percentage of variance in the mISS values for all analyzed 30-s bins as explained by the number of clusters. The percentage of variance is the ratio of the between-group variance to the total variance. The orange line delineates the point in the graph (3 clusters) in which an additional increase in the number of clusters does not yield a significant change in the percentage of variance explained.

(E) Histogram that plots the distribution of ISS values for all 30-s bins. The dotted lines represent the borders of the three clusters determined in (D). The “alert” group was further divided into three groups according to whether the ISS was greater than, equal to, or less than 0. See also Video S1.

(F) The distribution of overall mISS and mIBI. mIBI was correlated with mISS by Spearman’s rank correlation test ($\rho = 0.75$, $p < 0.001$).

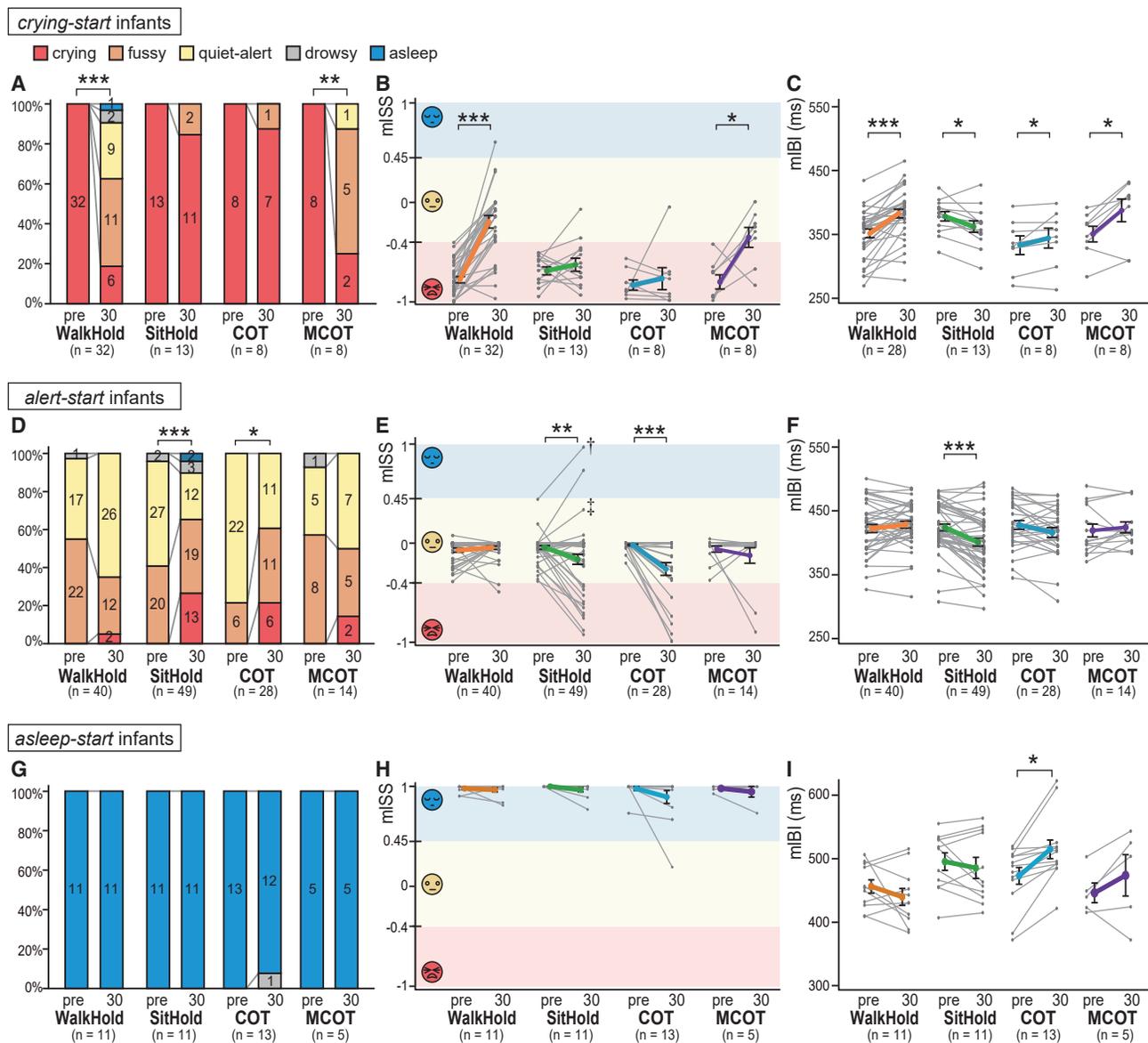


Figure 2. Effects of 30-s short maternal tasks on infant behavior and IBIs

30-s immediate effects of each maternal task on crying-start (A–C, $mISS < 0.4$ in the last 30 s in the preceding condition), alert-start (D–F, $-0.4 \leq mISS < 0.45$), and asleep-start (G–I, $mISS \geq 0.45$) infants.

(A, D, and G) The ratio of infants who were categorized into five groups according to their ISS values, induced by the four maternal tasks. Asleep, $mISS \geq 0.45$; drowsy, $0 < mISS < 0.45$; quiet-alert, $mISS = 0.0$; fussy, $-0.4 \leq mISS < 0.0$; crying, $mISS < -0.4$. Fisher's exact test was applied. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

(B, E, and H) Change in mISS by maternal tasks.

(C, F, and I) Changes in mIBI by maternal tasks.

Colored lines and black bars represent mean \pm SEM. Gray dots and lines represent the individual data. Two infants fell asleep within 1 min of the succeeding SitHold task after 5 min of WalkHold († and ‡, each symbol corresponds to those in Figure S2B; see Video S3). Wilcoxon signed-rank test was applied for mISS and mIBI. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

might not have separated the components of motion from maternal holding, such as standing, stepping, tapping, or swinging, which are often performed by parents and caregivers. Also, a short period of carrying was included in the transition from COT to SitHold and might have complicated the results. These issues should be specifically addressed to determine the effects of maternal holding on crying infants.

When an infant falls asleep in one's arms or in a stroller, parents would then want to put the infant to bed. However, this lay-down procedure often makes the infant alert again. In this study, 9 of 26 (34.6%) sleeping infants awoke by 20 s after the laydown, while the remaining 17 infants stayed asleep. In these successful laydowns, infants' IBI increased further (Figures 4B and 4C; Video S4). Transitions from WalkHold or SitHold to COT are often

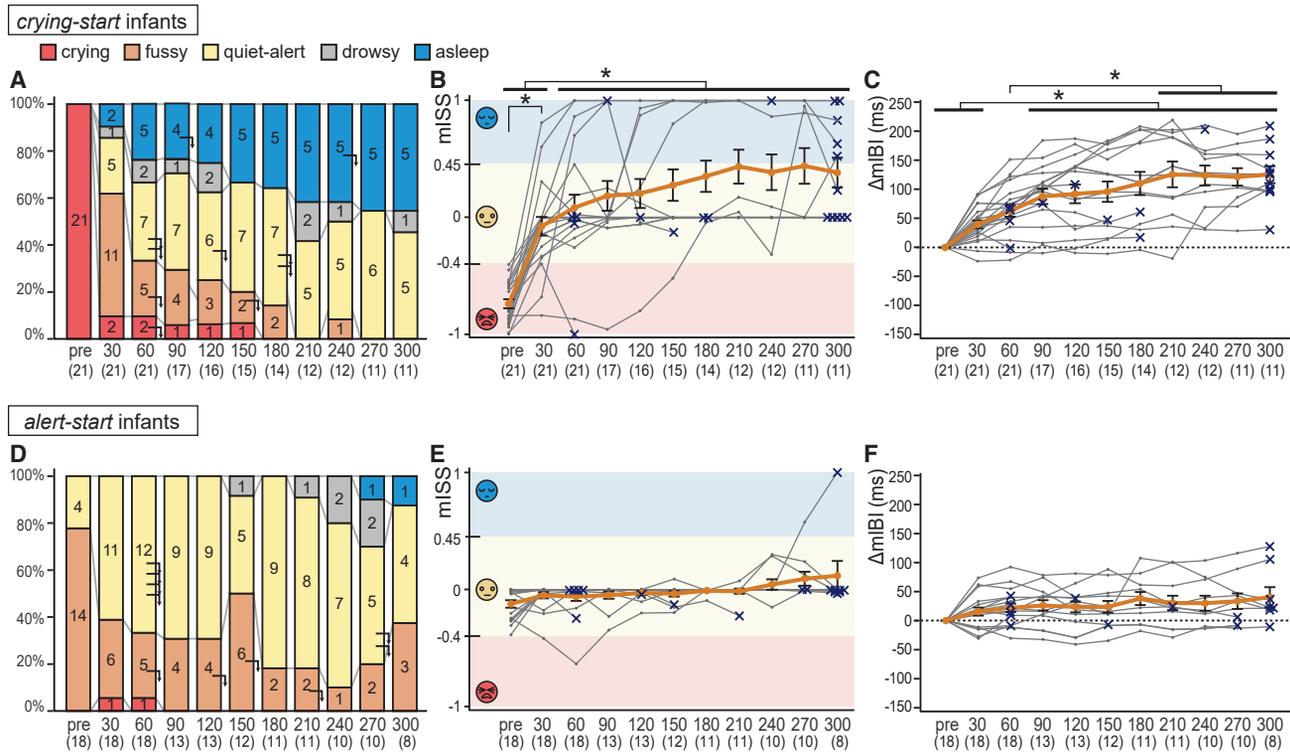


Figure 3. Effects of 5-min maternal carrying on crying and alert infants

Effects of maternal WalkHold longer than 60 s on crying-start (A–C) and alert-start infants (D–F). All the data points between 1- and 5-min task length were presented for transparency, with the number of samples in the parentheses.

(A and D) The ratio of infant status category. Each angled arrow represents an infant whose task was discontinued.

(B, C, E, and F) Mean \pm SEM of mISS (B and E) and Δ mIBI, the difference from the value in the “pre” bin (C and F).

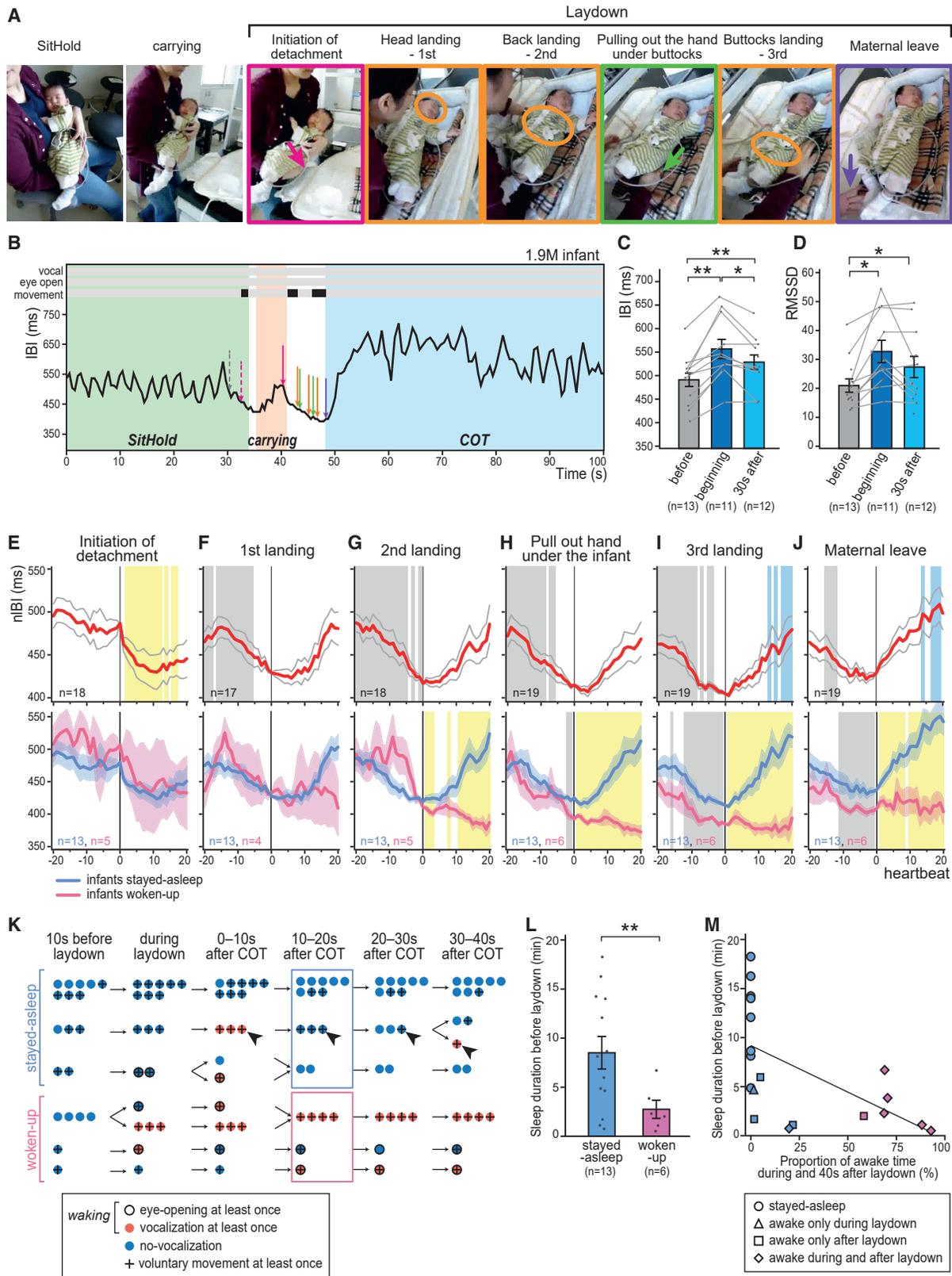
Gray lines represent individual data. Cross marks represent the terminal points of dropped-out infants. Multiple comparisons were done by Friedman’s test and a post-hoc Conover’s test for all pairs, with p values adjusted by Benjamini-Hochberg’s method, $*p < 0.05$. See also [Figure S2](#) and [Video S2](#).

associated with a head-down postural change and thus cause an autonomic IBI increase. However, this effect wanes after 30 s.^{26,27} Thus, we compared the infant IBIs averaged across three sequential time windows: (1) just before the laydown, (2) at the beginning of COT, and (3) at 30 s after the postural change. In 13 stayed-asleep laydown cases in which at least two windows were recorded without perturbation, the infant IBIs were indeed highest during the initial 20 s of COT and were still higher 30 s after the postural change, compared to the IBIs before laydown (Figure 4C). In addition, RMSSDs (root-mean-square of successive RR interval differences, an indicator of parasympathetic activity¹⁹) were also higher 30 s after the postural change than those before laydown (Figure 4D). Thus, after successful laydown, sleeping infants rested better in a still cot than in WalkHold or SitHold. Ethologically, a certain level of alertness during transport may be beneficial because maintaining a compact posture in rat and mouse pups is necessary for receiving effective carrying.^{14,15} Thus, it is reasonable that carrying elicits dual effects, calming in awake infants and alerting to a certain level in sleeping infants.

We next evaluated the sleeping infants’ physiological responses to each microevent during laydown (Figures 4A and 4B). IBIs from different infants were aligned at the initiation of each microevent, which was set to be the heartbeat count 0. Then these IBIs were normalized (nIBI) by the mean IBI at count

0 and were averaged across the infants. The resultant peri-event nIBIs from heartbeat counts -20 to $+20$ are shown in Figures 4E–4J (top panels). Zero-month-old infants showed blunted IBI responses to any microevent (Figure S4); thus, we used data from 1- to 7-month-old infants for the subsequent analyses. Examination of peri-event nIBIs against the baseline (heartbeat = 0) revealed that the most alerting (i.e., nIBI-decreasing) microevent during laydown of sleeping infants was the detachment from the mother (yellow shades in Figure 4E; pink solid arrow in Figure 4B). The subsequent first landing of the infant’s body did not significantly decrease the infant nIBI, nor did the later events (Figures 4F–4J). The final maternal detachment increased the infant nIBIs (blue shades in the upper panel of Figures 4I and 4J).

Separate analyses showed that, even during apparent sleep, infants responded physiologically to virtually any maternal action from the very beginning; when the mothers transitioned from SitHold to other conditions, the infant nIBIs significantly decreased by the initial replacement of maternal hand position on the infant body in preparation for standing (yellow shades in Figure S3D) and maternal leaning forward (Figure S3E), but not by standing up or walk initiation (Figures S3F and S3G). As the mothers walked with the infant, the infant nIBIs significantly decreased by maternal turning of the direction (Figure S3H) and by maternal stop-walking (Figure S3J), but not by the downward motion when the mothers sat down afterward



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(Figures S3K–S3M). As the mother picked up her sleeping infant from a cot, the infant nIBIs decreased most significantly by the first maternal touch to the infant's body (Figures S3N–S3Q). These data indicate that any transition of maternal actions can alert the sleeping infants.

Finally, we sought the key determinants of the sleep outcome after laydown. Since infant sleep/wake condition became relatively stable after 10–20 s from laydown, we assigned the infants who showed no eye opening or vocalization during the 10–20 s after laydown as stayed-asleep (13 of 19 infants) and the remaining infants as woken-up (6 of 19) (Figure 4K). This infant grouping stayed true until one hiccupping infant (arrowhead, Figure 4K) woke up 30 s after laydown. Comparing the peri-event nIBIs for these stayed-asleep versus woken-up groups showed that the two groups were not different at the initiation of laydown and diverged after the 2nd landing of infant body parts (Figures 4E–4J, bottom).

We initially hypothesized that slower maternal laydown could help maintain the infant's sleep. However, the total duration of laydown or intervals between any two microevents were not significantly shorter for woken-up infants than for stayed-asleep infants (Figure S3R). The mothers of woken-up infants rather took a longer time before the third landing, possibly in response to the infant's vocalizations or eye opening. Neither did the order of landing of infant body parts differ among these groups (Figures S3A–S3C; $\chi^2 = 6.04$, $df = 6$, $p = 0.42$).

The only significant group difference identified was how long the infants had been sleeping before the mother initiated the laydown (on average, 2.75 ± 0.92 and 8.52 ± 1.65 min for the woken-up and stayed-asleep group, respectively) (Figure 4L). Even among the stayed-asleep infants, eye opening or negative vocalizations were almost always observed if the laydown started within 5 min from the apparent sleep onset (Figure 4M). Thus, when the infant falls asleep in the caregiver's arms,

withholding the laydown until another 5 to 8 min have passed may reduce the probability of awakening the infant. This time window roughly corresponds to the length of stage-1 sleep (approximately 8 min in 4- to 5-month infants) at the sleep onset, which is associated with a low arousal threshold to a gentle touch or quietly closing a door.^{28,29} During this waiting period, 9 out of 13 stayed-asleep infants experienced SitHold, suggesting that sitting with a sleeping infant for a few additional minutes should be suitable for a successful laydown.

The present study is exploratory and needs confirmation in more specific experiments with larger samples. To retain the naturalistic mother-infant behaviors, we allowed flexibility on task length and did not strictly control vocal or non-verbal communications with the mother, which are known to affect infants' emotional state.^{30,31} Other parameters that may affect the results are motion velocity, frequency, the experiment timing (at night or nap time), infant crying habits, and sleeping routines. Also, the effects of carrying should be compared between mothers versus non-maternal caregivers because motion-based infant soothing is commonly performed by fathers, grandparents, and childcare professionals in several countries including Japan.

Nevertheless, this study provides a proof of concept that infant transport robustly reduces cry and potentially promotes sleep, and proposes a behavioral protocol, "5-min carrying, 5- to 8-min sitting before bed" for crying infants. For secure holding, caregivers should attach the infant body snugly to their own body and support the infant's head. Five-minute walking should be on a flat and clear passage and at a steady pace, preferably without abrupt stops or turns. With these precautions, this protocol can be safely performed within the range of regular parental care. It should be noted that, unlike most behavioral interventions for infant sleep difficulties,^{9,10} this protocol does not address any long-term improvement of sleep regulation. This

Figure 4. Infant IBI transitions as sleeping infants are laid in a cot

(A and B) An example of maternal laydown of a sleeping infant into a cot after SitHold. Colors of picture frames (A) and arrows on the infant IBIs (B) indicate pink, the mother starting to detach from the infant and putting the infant downward; orange, the landing of the infant's body parts; green, the mother pulling her hand from under the infant; and purple, the mother detaching from the infant completely. The dashed gray arrow is when the seated mother first changed her hand position on the infant, and the dashed pink arrow when the seated mother leaned forward in preparation for standing up. Black and light gray horizontal bars indicate the presence and absence, respectively, of infant vocalization, infant's eye opening, and infant voluntary body movements. Colored shades: orange, transient maternal walking; green, SitHold; light blue, COT. See also Video S4.

(C and D) Mean \pm SEM IBIs (C) and RMSSD (D) of infants who stayed asleep after laydown ($n = 13$), averaged over the 20-s segment just before the laydown (before), the initial 20 s of COT, excluding the transient unstable phase (beginning), and the 20-s segment starting at 30 s after the completion of postural change (30 s after). Three data points were excluded, two for overlapping with the next condition and another for infant jerky movements that resulted in unstable measurements. * $p < 0.05$, ** $p < 0.01$ by Friedman's test, and a post-hoc Wilcoxon signed-rank test with p values adjusted by Benjamini-Hochberg's method. (E–J, upper panel) Peri-event nIBIs shown in mean (red lines) \pm SEM (gray lines). Samples were excluded if any microevent was not clearly visible, causing variable sample sizes. Vertically shaded colors indicate significant differences ($p < 0.05$) compared to the nIBI at heartbeat = 0 (yellow, significant decreases after the microevent; blue, increases after the microevent; gray, differences existed before the concerned microevent) by Steel's test.

(E–J, lower panel) Peri-event nIBI differences between stayed-asleep (blue) and woken-up (pink) infants, expressed in mean \pm SEM. The infants who vocalized or opened their eyes in the period between 10 and 20 s after laydown (boxes in K) were classified as woken-up, and the rest were classified as stayed-asleep. Vertically shaded colors indicate significant differences ($p < 0.05$) between the two groups at each heartbeat (yellow, differences after the microevent; gray, differences before the microevent) by Mann-Whitney U test.

(K) Infant behaviors from 10 s before to 40 s after the laydown, coded for the presence or absence of vocalization, eye opening, and voluntary body movements. The time taken for laydown varied by individual. Each circle represents each infant. The arrowheads indicate an infant who started hiccupping after laydown, vocalized transiently at 36 s after laydown, then fell asleep again. Note that all the vocalizations observed in this figure were negative (cry or fuss), but not babbling or laughing.

(L) Infant sleep duration before the start of laydown. The woken-up ($n = 6$) versus stayed-asleep ($n = 13$) categories are identical to the boxes in (K). ** $p < 0.01$ by Welch's t test.

(M) Infant sleep duration before the laydown initiation, plotted against the proportion of awake time during and 40 s after the laydown. Circle, asleep during and after laydown; triangle, awake during laydown but asleep after laydown; square, asleep during laydown but awake after laydown; and diamond, awake both during and after laydown. The colors of the markers correspond to the categories in (L).

protocol instead provides an immediate calming of infant cry and may be useful especially on special occasions when the regular sleep routines, breastfeeding, or pacifiers are not effective or available.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.cub.2022.08.041>.

A video abstract is available at <https://doi.org/10.1016/j.cub.2022.08.041#mmc7>.

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AUTHOR CONTRIBUTIONS

N.O. and L.O. processed and analyzed data, with support from S.Y., A.T., E.M., A.B., and M.S. G.E. designed and carried out experiments with A.T. K.O.K. conceived and organized the study and designed and carried out experiments. K.S. and A.S. carried out experiments and analyzed data. N.O. and K.O.K. wrote the manuscript and prepared figures and videos with contributions from all the authors.

DECLARATION OF INTERESTS

Japanese Patent Application No. 2022-035010. The patent describes the principle to build a smartphone application that predicts minute-scale infant state

changes from the IBI and guides the caregivers for effective soothing and facilitation of infant sleep.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Summary statistics describing each data	This paper	https://doi.org/10.17632/xfbmzdw8wt.1
Software and algorithms		
MARS 8000 Holter analysis system	GE Healthcare	https://www.gehealthcare.com/courses/mars-ambulatory-ecg-analysis-system
CardioDay software	GETEMED	https://www.getemed.de/en/cardiology
Python version 3.10.1	Python Software Foundation	https://www.python.org
Premiere Pro CS5	Adobe	https://www.adobe.com/products/premiere.html
VideoPad v 10.68	NCH Software	https://www.nchsoftware.com/clipit/
SPSS Statistics 23	IBM	https://www.ibm.com/analytics/spss-statistics-software
R version 4.1.0	R Foundation	https://www.r-project.org
JASP 0.16	University of Amsterdam.	http://www.jasp-stats.org
Other		
SEER Light WP Holter ECG recorder	GE Healthcare	N/A
CardioMem CM 4000	GETEMED	https://www.getemed.de/en/cardiology#c5

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Kumi O. Kuroda (kumi.kuroda@a.riken.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- DATA: The full experimental data reported in this study cannot be deposited in a public repository because those include personally identifiable information such as the images of the human subjects. Summary statistics describing each data are deposited at Mendeley Data and are publicly available as of the date of publication. The DOIs are listed in the [key resources table](#). In addition, the de-identified IBI and ISS data generated in this study, along with several example video data are available from the [lead contact](#) upon reasonable request with a completed Materials Transfer Agreement.
- CODE: This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants

All experiments were approved by the Ethical Committee of RIKEN (Japan) and the University of Trento (Italy). The experiments were conducted essentially as described,¹⁴ with expanded participants. Briefly, typically developing infants of 0 to 7 months of age and their biological mothers were recruited, either in Japan or Italy. Mothers were fully informed about the contents of the study and agreed to participate before the experiments. Data from a total of 32 sessions with twenty one healthy infants (10 females, 11 males), who had a mean age of 3.34 months (SD = 2.01, SEM = 0.36, Min = 0.5, Max = 7.2) and their biological mothers who had a mean age of 35.81 years (SD = 5.29, SEM = 0.93, Min = 24, Max = 42, with the age of one mother undisclosed) were analyzed. Sessions for two 0-month-old infants (1 girl, 22 days old and 1 boy, 14 days old) were conducted at the participant's home. Two mother-infant dyads participated in multiple sessions on different days, and their first formal sessions were included. In some cases, the grandmother, the father, or an unfamiliar person with the infant also participated in place of the mother, but those cases were not included in the present

analysis. Experimental sessions were conducted either at the participant's home (53.13%, 17/32 sessions) or at a laboratory (15/32), as the mothers preferred. In both environments, special caution was taken to remove obstacles on the floor, to avoid stumbling of the participant with their infant. 15/32 sessions were recorded in Italy, and 17/32 were done in Japan. Mothers were asked to pick a time of the day when the baby was usually awake. Before starting the experimental session, all mothers were asked to sign a parental consent form and were informed that they could skip a particular task part at any moment, or later withdraw their child's participation in this study. None of the parents requested to withdraw.

METHOD DETAILS

ECG recording and extraction of IBI

ECG signal was acquired using a portable Holter (at 125 Hz, SEER Light WP Holter ECG recorder, GE Healthcare, or at 1024 Hz, CardioMem CM 4000, GETEMED). Five disposable electrodes (BlueSensor N, Ambu) were placed on the infant's chest to yield two parallel leads CM5 (positive electrode at V5), and one of these with a better recording quality was used for analyses. ECG data from these Holters were preprocessed using the MARS8000 Holter analysis system (GE Healthcare), or the CardioDay software (GETEMED) and a python program, both of which identified the QRS complexes and extracted a list of inter-beat-intervals (IBIs), which is the time elapsed between two consecutive R waves. IBI extraction errors were fixed by visual inspection and calibration.

Maternal tasks

Experimental sessions started approximately 1 to 2 hours after the last feeding to avoid strong autonomic effects from gastrointestinal function. Infants were first laid on a cot and were equipped with an ECG Holter. On some occasions, as expected, infants started fretting or crying during the application of ECG electrodes. For this reason, experimental procedures began after a 10-minute wait from the application of electrodes. Then, following the instructions, the mothers performed a random series of the following four tasks with their infant: WalkHold, the mother held the infant and walked; SitHold, the mother held the infant and sat; COT, the infant was laid in a cot; and MCOT, the infant was placed in either a mobile crib or a stroller and was moved in a back-and-forth manner. The mothers and experimenters stayed in the vicinity of the infants during COT or MCOT. Each task was set to be either 30-second (short condition) or 5-minute (long condition), with some variations inevitably caused by the timing of the instructions and maternal responses. The mothers were informed that they could interrupt the task at any time and move on to the next task, in case the mother felt uncomfortable with her infant's cry, or if the mother felt tired. The number of conditions in each experiment session varied across participants (Median = 19, Max = 38, Min = 2, SD = 10.32, SEM = 2.11). The median session duration was 39.00 minutes (Max = 90 min, Min = 11 min, SD = 21.18, SEM = 3.80). Aside from the sessions exceeding 60 minutes, which were performed by K.O.K., A.S., K.S. and their infants, the other participants had a duration of Max = 48 min.

The cot, or the platform on which the infant was laid were: a Moses basket ($n = 14$), a crib/cot ($n = 5$), a stroller ($n = 5$), a bed ($n = 3$), a cradle ($n = 1$), a rocking chair ($n = 1$), a swing crib ($n = 1$), a sofa ($n = 1$) or a bedding ($n = 1$). Data from six infants were used as the MCOT condition: with a stroller or a movable crib/cot (back-and-forth horizontal movement in the infants' cranial-caudal direction, $n = 4$), with a rocking chair-type crib ($n = 1$), or with a swing crib (sideways swing with yaw rotation, $n = 1$). As these different devices did not appear to yield different results, the data were combined for analyses. The stepping frequency of WalkHold ranged from 0.77 to 2.67 Hz, and the frequency of rocking motion of MCOT was 0.80 to 2.27 Hz, both comparable to or slightly slower than normal walking of women (1.48–2.48 Hz³²). The highest stepping frequency of WalkHold was attempted by K. O. K., because it has been reported that higher vertical rocking in the range of 0.5 Hz to 1.5 Hz reduced infant arousal.³³ Note that this was not a high *velocity* of walking, and one foot was always kept in contact with the floor (i.e., no running).

Video recording and synchronization with IBI

All sessions were videotaped by a handheld camera, and some sessions had an additional camera from a fixed position, to capture the participants' behaviors, facial expressions, body movements, and vocalizations as much as possible. The ECG and its recorded video were synchronized by pressing the event button on the Holter to mark the time during ECG recording. Synchronization and behavioral coding were done at each IBI level, using the software Premiere Pro CS5 (Adobe) or VideoPad (NCH Software).

Maternal task coding

The coding for the start and the end moments of maternal tasks were defined as follows. WalkHold was when the mother started to move her legs, until either when she stopped both legs, or right before she started to lean forward for sitting or for laying down her infant. SitHold condition was coded as starting when the mother sat down and stabilized the angle of her upper body tilt, and ending right before she started to lean forward again for standing up. COT condition was coded as starting after the first complete detachment of maternal hands from the infant and lasted until the moment before she touched the infant again. For MCOT, meeting the COT criteria was a prerequisite, and it was the time between the start and end of cot movements by the mother.

Infant status scoring

The behavioral sleep/wake state categorizations have been described previously, but without specific quantitative criteria or agreement among researchers.^{20,34} Rigorous manuals have been published to categorize different sleep states in infancy, but not for awake states.^{35,36} Moreover, while polysomnography has been established to categorize micro and macro structures of sleep

even in early infancy, recording reliable electroencephalography measurements during dynamic alteration of maternal holding, walking, and laydown was not feasible. In several preparatory sessions, we have also tried additional measures including electromyography, respiratory chest movements, skin conductance, and foot temperature (known to increase before sleep onset) of the infants. However, attaching many pieces of equipment securely onto the infant's body induced infant cry, and these attachments and cables disturbed task transitions and natural behaviors of the mother. Furthermore, the resultant recordings were often too noisy because of the activities of the dyad. Therefore, in this study, we focused on measuring the ECG and behavior with minimal additional stress caused by measurements. To quantitatively and continuously score the infant state ranging from cry to sleep from infant behaviors, infant negative vocalizations at each heartbeat were coded separately for short-bouts (less than 0.5 sec, which correspond to apparent fuss or fretting) or long-bouts (0.5 sec or longer, for apparent crying). Infant eye state at each heartbeat was first coded for the following possibilities: at least one eye being open, both being closed, or neither being visible in the video. For moments where the video did not show the infant eyes, the eye state was interpolated with the nearest available eye state. Then we created the operational scoring of ISS at each heartbeat from -1 to 1 : ISS = 1 , eyes closed and no negative vocalizations (presumptive sleep); ISS = 0 , at least one eye being open and no negative vocalizations; ISS = -0.5 , emitting negative vocalizations shorter than 0.5 sec; and ISS = -1 emitting negative vocalizations longer than 0.5 sec. It should be noted that infants close their eyes during hard crying.

These coding rules worked well to represent infant behavioral states, except for the following three cases; first, when infants were crying hard, infants often took long breaths which produced no sounds ([Video S1](#)), and this period could be erroneously coded as the “quiet alert” state. Thus, to correctly account for breathing during crying, the ISS was considered to be -1 during the voiceless breathing periods that intervened in the cries and which lasted less than 1 second. Second, when the infants started to cry, they sometimes closed their eyes tightly and showed a grimace without vocalization, and this period was erroneously coded as the asleep state (ISS = 1). Therefore, eye-closed periods for 2 seconds before and after the negative vocalization was coded as ISS = 0 , and not ISS = 1 . Third, eye blinks could also be erroneously coded as sleep (ISS = 1) state, so eye-closed periods less than 1 second were coded as ISS = 0 .

Binning of conditions

In this analysis, a condition denotes a data segment with continued execution of any of the four maternal tasks, of which the starting moment was visibly recorded. Any condition that was shorter than 25 seconds, or any condition which had a gap larger than 30 seconds from the end of the preceding condition, was excluded from the analysis. The conditions were then sliced into 30-second time bins that do not overlap, up to a maximum of 300 seconds (five minutes). If the last bin of a given condition was longer than 25 seconds, it was regarded as approximately 30 seconds and was included in the analysis (45 cases/266 conditions), whereas a final bin that was shorter than 25 seconds was discarded, and the second last bin was regarded as the last 30-sec bin for the given condition. With the above screening criteria, a total of 837 bins from 266 conditions were achieved from the samples. Analyzed conditions were paired with a “pre” bin as the baseline, which was the last continuous 30 seconds of a preceding maternal task. For each bin, mean IBI (mIBI) and mean ISS (mISS) were calculated.

Infant state classification

To empirically categorize the infant states, we performed a *k*-means cluster analysis³⁷ using 763 mISSs analyzed in this study. This analysis requires that *k*, the number of expected clusters, must be specified a priori, thus we used the elbow method as a heuristic to estimate the optimal number of clusters.³⁸ This method is based on the plot of the ratio of the between-group variance to the total variance (total sum of squares) as a function of cluster number ([Figure 1D](#)). An inflection in this curve (the “elbow”) reflects the number of clusters that account for a substantial fraction of the variance. Thus, the location of the elbow is used to delineate the appropriate number of clusters within a given dataset. The resultant categorization of the mISSs is presented in [Figure 1E](#). For visualization, the alert state was further subdivided into fussy (or active alert)²⁰ ($-0.4 \leq \text{mISS} < 0$), quiet alert (mISS = 0), and drowsy ($0 < \text{mISS} < 0.45$) (see [Video S1](#)).

Analyses of maternal task effects on infant

To analyze the immediate effects of four maternal tasks on infant IBI and ISS, the initial (up to 30 seconds from the start of a given maternal task) bins were compared to the “pre” bin, the last 30-second bin in the preceding condition. As sessions contained multiple task transitions, a maximum of two of the earliest conditions were accepted for analysis per every maternal task-transition type (e.g., WalkHold to SitHold) per each mother-infant dyad. For longer-term effects of four maternal tasks on infant mIBI and mISS, conditions that lasted longer than 2 bins (more than 55 seconds from the start of the task) were used. The number of samples from the same participant was restricted to be a maximum of two for a given type of transition (e.g., WalkHold to SitHold) in the following way: the conditions with a longer duration were selected first, and if there were conditions with equal durations, the earlier ones were selected. For individual plots of mIBI and mISS ([Figure S2](#)), all data were used, including data from three or more conditions having the same transition type in the same session.

Participants for laydown analysis

The laydown procedure of sleeping infants was analyzed in 26 samples, in which the initiation of laydown was accompanied by a preceding 10 seconds of undisturbed infant sleep-state, with full eye closure and no vocalization. Seven non-neonate infants (3 girls

and 4 boys, 1–7 months old) provided 19 samples of being laid down by their mothers, and two neonate infants (1 girl, 22 days old and 1 boy, 14 days old) provided 7 samples of being laid down by their mothers (6 cases) and grandmother (1 case).

Infants' vocalization and eye state were coded, similar to the ISS coding, from 10 seconds before to 40 seconds after the laydown, at each heartbeat. In addition, the presence of infants' voluntary body movements, including any small but noticeable movement (e.g., of a finger), were coded, although the extent of visibility of the infant body varied in the videos. Even if the voluntary movement was present, infants were regarded as staying asleep as long as their eyes stayed closed, and they did not vocalize.

Infant body landing

In most cases when mothers laid the infant down, mothers held the infant with one hand behind the neck and shoulders, and the other hand under the buttocks or thighs. The mother laid the infant on its back in 18/19 samples of the 1-month-old and older infants, and 7/7 of the neonate samples, whereas one infant was laid with a sideways landing, later to be turned on its back. The order of infant body parts that were landed was classified into three parts: head, back (including shoulders), and buttocks. The shoulders (upper trunk) and the back (middle trunk) were combined because these happened closely after each other, except for one case where the mother used a baby sling and had to drag the sling out from under the infant's body. The orientation of the head when it landed was either on the occipital side in 11/19 cases of the older infants and 4/7 in neonates, or on the temporal side in 5 of the older samples (3 in neonates), and the orientation was invisible in 2 cases.

IBI transition at each microevent

The influence of laydown microevents on the sleeping infants' IBIs was analyzed as follows. Peri-event IBIs (± 20 heartbeats from the microevent) were aligned to the moment of microevent initiation as heartbeat = 0, and were normalized by subtraction to match the mean IBI of the microevent sample at 0 heartbeat. Normalized IBIs (nIBIs) at each heartbeat before and after the microevent were compared to the baseline nIBI at heartbeat = 0. To examine the turning point for the outcome between the infants continuing to sleep or becoming awake, the peri-event nIBIs were compared between these groups at various microevents during laydown.

Detection of sleep-onset time before laydown

To examine the effect of sleep duration on the sleep/wake outcome, the interval between the initiations of infant sleep and laydown was calculated. The timing of the infants' sleep onset was defined as the initiation of the behavioral sleep state, satisfying both of eye closure and no vocalization.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

All statistical analyses were conducted using the statistical software SPSS Statistics 23, R version 4.1.0, and JASP 0.16 (<http://www.jasp-stats.org>). Shapiro-Wilk test for normality, Levene's test for variance, *k*-means clustering using elbow method, Spearman's rank correlation test, Fisher's exact test, chi-square test, Student's *t*-test, Welch's *t*-test, Mann-Whitney U test, paired *t*-test, Wilcoxon signed-rank test, Friedman's test, Steel's test, and Conover's test were used. Multiple comparisons were conducted with *p*-values adjusted by Benjamini-Hochberg's method. Error bars are mean \pm standard error of mean unless otherwise specified. All the statistical details can be found either in the figure legends or results.

Linear mixed model (LMM) analysis was conducted with *lme4* and *lmerTest* packages on R software. Of note, although a fixed effect of sleep state at the beginning of the session ($t = -2.65$, $df = 19.8$, $p = 0.015$) was significant for mISS of alert-start infants, this result was derived from four repeated cases of one mother-infant dyad.