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Article

Infant Calming Responses during Maternal Carrying in Humans and Mice

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Summary

Background: Mother-infant bonding is the earliest and most critical social relationship of mammalian infants. To promote this bond, infants have innate behaviors to seek maternal proximity and protest upon separation via communication with the mother vocally and through body movement. However, the physiological mechanisms regulating these infant behaviors remain largely undefined.

Results: Here we show a novel set of infant cooperative responses during maternal carrying. Infants under 6 months of age carried by a walking mother immediately stopped voluntary movement and crying and exhibited a rapid heart rate decrease, compared with holding by a sitting mother. Furthermore, we identified strikingly similar responses in mouse pups as defined by immobility and diminished ultrasonic vocalizations and heart rate. Using pharmacologic and genetic interventions in mouse pups, we identified the upstream and downstream neural systems regulating the calming response. Somatosensory and proprioceptive input signaling are required for induction, and parasympathetic and cerebellar functions mediate cardiac and motor output, respectively. The loss of the calming response hindered maternal rescue of the pups, suggesting a functional significance for the identified calming response.

Conclusions: Our study has demonstrated for the first time that the infant calming response to maternal carrying is a coordinated set of central, motor, and cardiac regulations and is a conserved component of mammalian mother-infant interactions. Our findings provide evidence for and have the potential to impact current parenting theory and practice, since unsoothable crying is the major risk factor for child abuse.

Introduction

Mammalian infants require continuous parental care for survival and psychosocial development [1]. To achieve this, infants have the innate drive to seek for maternal proximity and protest upon separation, via communication with the mother vocally and through body movement [2, 3]. These active contributions by infants are as essential as parental care-giving behaviors for the establishment of the mother-infant bond. Yet, despite their critical importance, the physiological and sensorimotor mechanisms regulating these infant behaviors are poorly understood, especially those mediating positive responses in infants such as calming and relaxation in response to maternal care.

Altricial mammalian neonates have limited ambulatory ability and require maternal carrying for transportation. In humans, carrying of a baby in the arms, in a sling, or in a stroller while walking is also commonly performed as a soothing measure. However, the calming effect of infant carrying has been controversial in the previous studies [4–7], all of which measured the total amount of crying and carrying within 1 hr or longer relying on parental diaries, rather than direct observation, and tried to correlate these variables. Moreover, no distinction was made in these reports between mobile carrying and simple holding without movement. In this study, we focused on the real-time, acute effects of maternal carrying, with audio-video monitors and electrocardiogram, which enabled us to record infant behavioral and physiological responses at a subsecond time scale. Using these methods, we investigated infant responses to maternal carrying and the sensorimotor mechanisms in both human infants and mouse pups, to elucidate infant cooperative behavior during maternal carrying in mammals.

Results

Calming Responses to Maternal Carrying in Human Infants

The behavior, vocalization, and electrocardiogram (ECG) of human infants were monitored during behavioral tasks that consisted of lying in a crib (*CRIB*), held by the mother who was sitting on a chair (*holding*), or held by the mother who was walking continuously (*carrying*) (Figure 1A). In initial experiments we tested various durations and combinations of the three conditions (Figures S1A and S1B available online), and we found that all measures of the infant (crying, body movement, and heart rate) generally increased during the *CRIB* condition while they decreased during *carrying*. The *holding* condition had intermediate effects between *CRIB* and *carrying*. During *holding-carrying* repetitions (Figure 1B and Movie S1), the interbeat interval (the inverse of heart rate) increased rapidly at the start of *carrying* and returned to the previous level after the start of the next *holding*. Therefore, we focused on the *holding-carrying* transition period in this study.

Twelve healthy infants, 1 to 6 months of age (mean = 3.08 ± 0.51 months, six females and six males) were recruited for the

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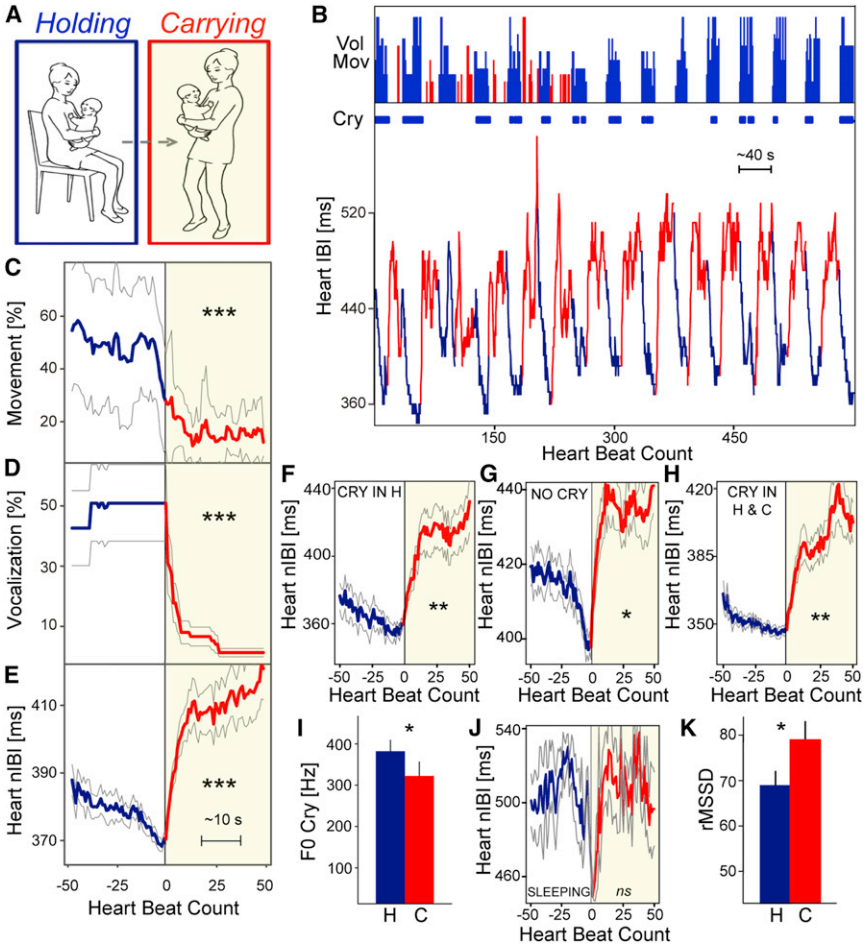


Figure 1. Carrying-Induced Calming Responses in Human Infants

(A) Behavioral task of *holding* (blue throughout this paper) and *carrying* (red with yellow background throughout this paper) by the mother-infant dyad.

(B) An example of the task consisting of repetition of *holding* and *carrying*. Each condition lasted approximately 20 s. Amount of voluntary movement, presence of crying, and the interbeat interval (IBI) of the infant are presented.

(C–E) Time course of voluntary movements (C), crying (D), and the normalized IBI (nIBI) (E) of the *holding-carrying* transition of 12 human infants under 6 months of age. Event-triggered averaging of the effect of *holding-carrying* transition on the behavior and IBI was performed with the last heartbeat in the *holding* as a trigger (x = 0), over the time segment of −50 to +50 heartbeats from the trigger. The movement, crying, and IBI were averaged at each beat for each participant and were then averaged for all participants. Time-scale bars (s) in this figure were calculated from the average IBI. Maternal cadence (footsteps) per min during *carrying* was mean = 79.4, SD = 14.6, with a range of approximately 60–120 footsteps per min. Prior to data analysis, we tested the effect of possible covariates (child sex and age, maternal age, cadence) on the dependent variables (IBI, cry, voluntary movement) and found no significant correlations for any of the dependent variables; therefore, these were not considered as covariates in the subsequent analysis.

(F–H) Time course of the nIBI of the *holding-carrying* transition with more than 50% crying in *holding* but no cry in *carrying* (n = 13; F), without crying (n = 25; G), and with more than 50% crying in both *holding* and *carrying* (n = 6; H).

(I) The fundamental frequency (F0) of crying in the infants in (H).

(J) Time course of the nIBI when infants were sleeping (n = 7) throughout the *holding-carrying* period.

(K) rMSSD (heart rate variability index of parasympathetic activity) during the *holding* and *carrying*.

*p < 0.05, **p < 0.01, ***p < 0.001. All numerical data are shown as mean ± SEM. See also Figure S1, Table S1, and Movie S1.

main experiments. Ten minutes after holter electrode application, their mothers were asked to perform one of the three conditions (*CRIB*, *holding*, or *carrying*) sequentially, for 30 s each, in a randomized order indicated by the experimenter. Subsequently the data of awake infants during the time period of *holding* followed by *carrying* were collected and analyzed. Infants were crying in about half of the initial *holding* condition (Figure 1D). When the mother stood up and started walking, the infants significantly halted voluntary movements [*t*, Student's t test: $t_{(11)} = 5.21$, $p < 0.001$] and crying [$t_{(11)} = 4.01$, $p < 0.001$] (Figures 1C and 1D and Table S1). Moreover, we found that the infants' interbeat interval was elevated immediately after the start of *carrying* [increase (mean ± SEM) = 7.39% ± 0.04%, time constant = 3.16 s; corresponds to eight heartbeat counts; $t_{(11)} = 5.08$, $p < 0.01$] (Figure 1E).

Infant crying strongly increases physical activity and significantly alters the respiratory pattern. Therefore, the increase of interbeat interval may be secondary to the cessation of crying. To address this question, we classified each *holding* to *carrying* transition episode into four groups: (1) the infants were not crying throughout (n = 25), (2) the infants were crying more than 50% of the time during *holding* but not during *carrying* (n = 13), (3) the infants were crying more than 50% of the time during both *holding* and *carrying* (n = 6), and (4) other (excluded from this analysis; n = 8). The interbeat interval changes were then analyzed within these groups (Figures 1F–1H). The largest change was observed in the group of infants who cried only during *holding* [$t_{(12)} = 4.70$, $p < 0.01$; Figure 1F]. However, the rapid interbeat interval increase due to *carrying* was also observed in infants who were not crying during *holding* [increase (mean ± SEM) = 3.54% ± 0.04%; $t_{(24)} = 2.3$, $p < 0.05$] (Figure 1G), indicating that at least some portion of the cardiac effect was independent from the cessation of crying. For group 3, infants were crying 96.7% ± 8.2% (mean ± SD) during *holding* and 65.8% ± 17.8% (mean ± SD) during *carrying*, and the interbeat intervals were significantly increased after the start of *carrying* [$t_{(6)} = 2.24$, $p < 0.05$] (Figure 1H). A modest decrease in the cry fundamental frequency, F(0), was observed after the start of *carrying* in the latter group of infants [$t_{(6)} = 6.10$, $p < 0.001$] (Figure 1I). A separate analysis showed that the elevation of interbeat interval due to *carrying* was not observed in sleeping infants (Figure 1J and Table S1), suggesting a ceiling effect or the involvement of infant cognitive function or awareness. In both awake and sleeping infants, the transient decrease of interbeat interval was observed (Figure 1G, from −9 to 0 heart beat count; Figure 1J, from −4 to 0 heart beat count). The start time of this decrease in interbeat interval roughly corresponded to the beginning of maternal

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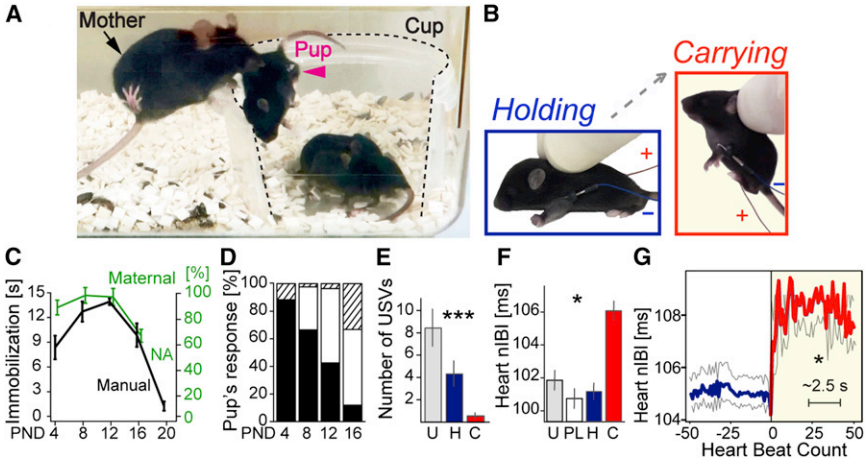


Figure 2. Carrying-Induced Calming Responses in Mouse Pups

(A) The behavioral task of maternal rescue, in which a C57BL/6 mother (arrow) rescued her pup (arrowhead) from a transparent plastic cup fixed in the home cage.

(B) Manual *holding* (blue) and *carrying* (red) of a mouse pup with two electrodes (+ and −) for ECG recording.

(C) The mouse pup's immobilization during maternal carrying (green line, n = 30 per age; shown as a percentage of total time because the maternal rescue time varied for each pup) and immobilization time (s) during manual *carrying* for 15 s (black line, n ≥ 18 per age).

(D) The pups' behavioral responses during maternal rescue from a cup. Immobilization with extended limbs (black), immobilization with flexed limbs (white), or voluntary movement (stripe) (n = 30 per age). Configural frequency analysis showed a decrease in pups that were immobilized with extended limbs and an increase in pups that were immobilized with flexed limbs with age (χ^2, chi-square test: $\chi^2 = 9.02$, $p < 0.001$).

(E) Number of USV emissions during *undisturbed* (U), *holding* (H), and *carrying* (C) of PND 7 pups (n = 18).

(F) nIBIs during *undisturbed* (U), *platform-lift* (PL, the pups were lifted on a platform to avoid direct contact by the experimenter), *holding* (H), and *carrying* (C) of PND 10 pups (n = 20).

(G) Time course of the nIBI during *holding-carrying* transition of PND 10 mouse pups (n = 20). Time-scale bars (s) were calculated from the average IBI.

*p < 0.05, **p < 0.01, ***p < 0.001. All numerical data are shown as mean ± SEM. See also Figure S2, Movie S2, and Movie S3.

preparatory movements for standing up, such as repositioning of the infant. Therefore, these transient dips of interbeat interval could be explained by an infant defensive reflex (acceleration of the heart rate caused by becoming alert due to sudden and intense stimulation) in combination with a baroreflex caused by lifting up by maternal standing similar to the head-up tilt test. However, the sustained elevation of interbeat intervals due to *carrying* in awake infants could not be explained by any known cardiac vagal reflex, including the orienting reflex (brief period of heart rate deceleration by mild sensory stimulus) [8], suggesting that carrying evokes a sustained heart rate reduction in concert with the rapid behavioral changes in human infants via a novel mechanism.

Heart rate variability analyses revealed that the index of parasympathetic activity rMSSD (square root of the mean of the sum of squares of differences between adjacent interbeat intervals over the length of the analysis) [9] was significantly higher during *carrying* than during *holding* [$t_{(12)} = 2.07$, $p < .001$] (Figure 1K). These data suggest that infants were more relaxed during *carrying* than during *holding*, not only behaviorally but also physiologically.

Calming Responses to Carrying in Mouse Pups

Our findings with human infants are evocative of the response to maternal oral transport observed in altricial mammalian young such as cats and squirrels. The carried young often adopt a characteristic compact posture with their hind legs drawn up [10, 11]. However, no experiments have been performed to directly measure the carrying-induced behavioral calming and concomitant physiological changes in the carried young. We hypothesized that, similar to human infants, mouse pups will also have behavioral and physiological responses that accompany the change in posture in response to maternal carrying. To test this hypothesis, we designed a naturalistic behavioral task in which mother mice rescued pups placed in a cup (Figure 2A and Movie S2). Every day during postnatal days (PNDs) 4–16, three pups were taken from the nest and were placed into the cup to induce maternal rescue. All the pups were retrieved within 15 min. The pups' responses to the maternal retrieving and maternal rescue time were measured via frame-by-frame video analysis. We found that during maternal *carrying*, pups maintained an immobile and compact posture (Figures 2C and 2D) for the majority of the task duration. The calming response was no longer evident at PND 20, when the pups were weaning and able to escape from the cup by themselves (data not shown). Similar responses could also be induced by an experimenter's manual carrying, by holding the small amount of skin at the nape of the neck, mimicking the maternal oral grasp (Figures 2B and 2C and Movie S3).

Next we examined whether the calming response of carried mouse pups was similar to that of human infants. Rodent pups emit ultrasonic vocalizations (USVs) at 40–80 kHz when they are separated from their mother and littermates [12]. Mother mice approach digitally recorded pup USVs, but not other synthesized ultrasounds [13], suggesting a comparable function of USVs with cries in human infants [14]. We found that *carrying* rapidly reduced pups' USV emission when compared with the *holding* or *undisturbed* conditions [*F*, Fisher's ANOVA: $F_{(2,41)} = 21.5$, $p < 0.001$] (Figure 2E). Moreover, similar to human infants, *carrying* had a significant effect on increasing the pups' interbeat interval [increase (mean ± SEM) = 4.73% ± 0.08%, time constant = 0.55 s; corresponds to 5 heart beat counts; $t_{(19)} = 3.2$, $p < 0.001$] (Figures 2F, 2G, and S2). The increase of interbeat interval did not occur when the pups were held in the same manner but without being lifted, or when the pups were lifted together with an underneath plastic plate (platform) (Figure 2F). Therefore, in mouse pups, *carrying* induced calming responses similar to those in human infants, even though maternal carrying methods differed.

Sensory Mechanisms Inducing the Calming Responses

We next examined the sensory inputs required for induction of the calming response in the mouse model. In humans, it has been suggested that the maternal touch [15] and rocking (vestibular-proprioceptive stimulation) [16] have calming effects in infants. The fact that the experimenter's grasp can induce the calming response in mice suggested that in

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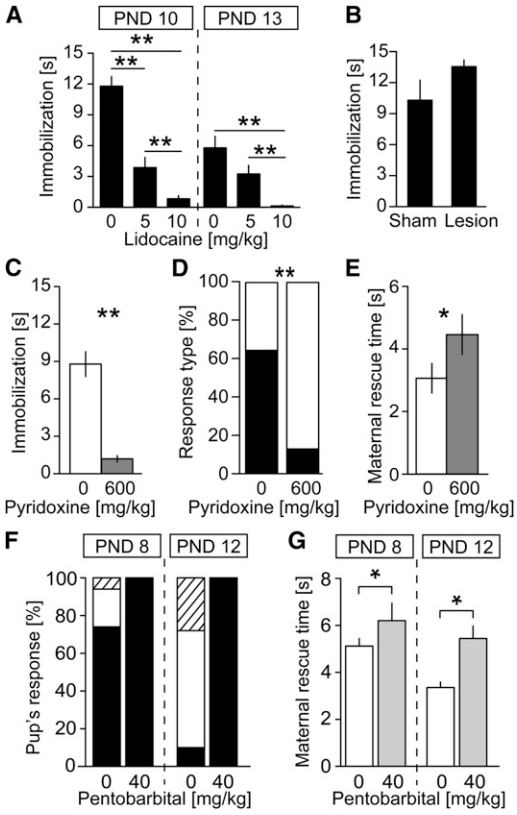


Figure 3. Sensory Input Mechanisms and Functional Significance of the Calming Response to Carrying in Mouse Pups

(A) Immobilization time (s) induced by manual carrying in the pups that received an injection of lidocaine diluted with saline at 0 (saline), 5, or 10 mg/kg 10 min prior (n $\geq$ 12 in each condition).

(B) Immobilization time (s) induced by manual carrying in the sham operated (n = 10) and vestibular lesioned (n = 11) pups. The pups received the operation at PND 10 and were tested at PND 13. No significant difference was found between the two groups (p = 0.14). Vestibular dysfunction in lesioned pups was verified by three behavioral tasks (Table S2).

(C) Immobilization time (s) induced by manual carrying in the pups that were treated by saline (0) or 600 mg/kg pyridoxine (600) from PND 10 to 12 twice a day, to induce transient proprioceptive dysfunction [17] (n = 16 each).

(D) The response of PND 13 pups treated with saline (n = 14) or pyridoxine (n = 16) during the maternal rescue. Black, immobilization; white, voluntary movements.

(E) Maternal rescue time (s) for the pups injected with saline (n = 14) or pyridoxine (n = 16).

(F) The response of PND 8 and 12 pups (n = 20 per group) injected with a saline (0) or 40 mg/kg pentobarbital (40) 10 min prior to the test session. Black, immobilization with extended limbs; white, immobilization with flexed limbs; stripe, voluntary movements. The configural frequency analysis showed the significant difference between the treatment groups (χ^2 = 65.29, p < 0.001).

(G) Maternal rescue time (s) for the pups treated with saline or pentobarbital (n = 40). With the general linear model with repeated-measurements, the differences for the retrieval time emerged as a main effect for age [$F_{(3,40)}$ = 1.98, p < 0.05] and for group [$F_{(3,40)}$ = 15.48, p < 0.001]. No interaction effects emerged.

*p < 0.05, **p < 0.01. All numerical data are shown as mean ± SEM. See also Table S2.

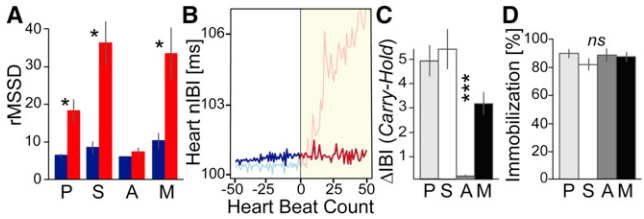


Figure 4. Autonomic Regulation of the Calming Response to Carrying in Mouse Pups

(A) rMSSD (heart rate variability index of parasympathetic activity) during the *holding* and *carrying* transitions of PND 10 mouse pups, pretreatment (P, n = 40) and treated by saline (S, n = 20), 2 mg/kg atropine (A, n = 20) or 2 mg/kg metoprolol (M, n = 20).

(B) Time course of nIBI at the transition from *holding* to *carrying* of saline- (lighter line, n = 20) versus atropine- (darker line, n = 20) treated pups.

(C and D) The nIBI changes between *holding* and *carrying* (C) and the percentage immobility during *carrying* (D) of PND 10 mouse pups described in (A).

*p < 0.05, **p < 0.01, ***p < 0.001. All numerical data are shown as mean ± SEM. See also Figure S3 and Table S3.

mouse pups tactile sensation of the skin and the sense of "being suspended" (passive transport of the body) are also important. On the other hand, olfactory, auditory, and visual inputs did not seem to be required to elicit the calming responses to manual *carrying* of mouse pups (it should be noted that the eyelids of the mouse pups younger than PND 14 are not fully open). To test the working hypothesis that maternal-like touch and vestibular-proprioceptive stimulation are also important for the mouse calming response, we first investigated the role of tactile sensation and found that the immobilization response of pups upon manual carrying was significantly reduced by local anesthesia on the skin of the neck in a dose-dependent manner at PND 10 [$F_{(2,\ 26.28)}$ = 61.72, p < 0.001] and PND 13 [$F_{(2,\ 18.56)}$ = 18.53, p < 0.001] (Figure 3A). We then examined the role of vestibular sensation and could not find any abnormality in the immobilization response in PND 13 pups that had received bilateral surgical damage to the vestibular organ (labyrinthectomy) at PND 10 [$t_{(10.95)}$ = 1.59, p = 0.14] (Figure 3B). Next, we tested pharmacological deprivation of proprioception by toxic overdose administration of pyridoxine (vitamin B6) [17]. Immobilization of PND 13 mouse pups upon manual and maternal carrying was significantly attenuated by proprioceptive dysfunction [$t_{(18)}$ = 7.2; p < 0.001] (Figures 3C and 3D). These results suggested that tactile sensation and proprioception are the primary sensory inputs required for eliciting calming, at least in mice.

Neural Mechanisms of the Cardiac and Motor Responses

We next investigated the output mechanism of each component of the carrying-induced calming response. Consistent to our finding in human infants, heart rate variability index rMSSD was significantly higher during *carrying* than during *holding* in mice [$F_{(1,75)}$ = 85.4, p < 0.001] (Figure 4A and Table S3), suggesting the involvement of parasympathetic nerve activation. To test the role of the autonomic nervous system directly, we observed mouse pups before and after intraperitoneal injection of muscarinic receptor antagonist atropine, postsynaptic β-adrenergic receptor antagonist metoprolol, or saline as a vehicle. The carrying-induced interbeat interval increase was abolished by atropine [no differences between *carrying* and *holding*, $t_{(19)}$ = 1.1, not significant] (Figures 4B, 4C, and S3), suggesting that the cardiac effect of carrying was largely dependent on parasympathetic activity. On the other hand, the immobility response of *carrying* was independent from the autonomic nervous system or from the heart rate reduction (Figure 4D). Although the behavioral and physiological components in response to carrying are expressed in

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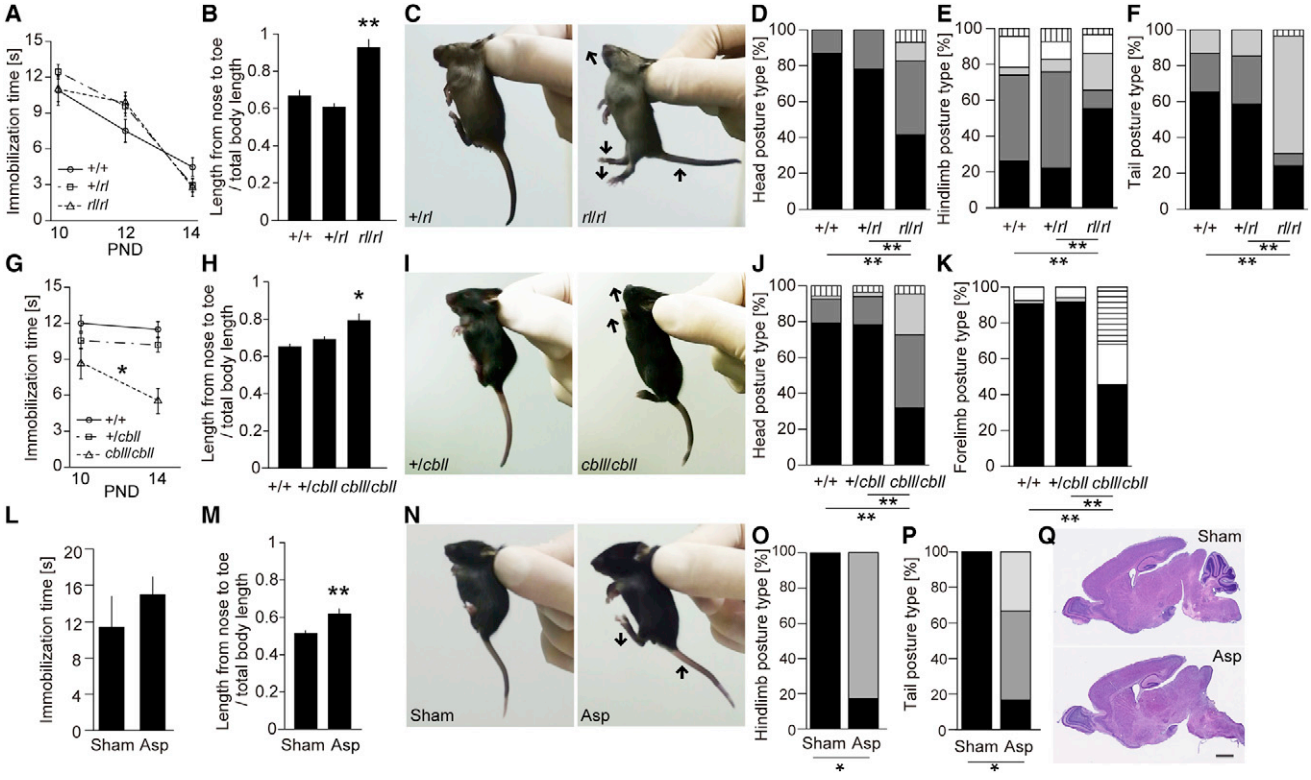


Figure 5. Aberrant Responses to Carrying in Genetic Mutant Pups and in Pups with Surgical Removal of the Cerebellar Cortex

(A–F) The responses in wild-type (+/+, n = 23), heterozygous (+/*rl*, n = 41), and homozygous (*rl*/*rl*, n = 29) *reeler* mutant pups during manual carrying. Immobilization times (A), body length (B), the representative posture during manual carrying (C), head direction (D), hind-limb posture (E), and tail direction (F) in *reeler* mutant pups are shown.

(G–K) The responses in wild-type (+/+, n = 53), heterozygous (+/*cbll*, n = 83), and homozygous (*cbll*/*cbll*, n = 22) *cerebelless* mutant pups during manual carrying. Immobilization times (G), body length (H), the representative posture (I), head direction (J), and forelimb posture (K) in *cerebelless* mutant pups are shown.

(L–Q) The responses of the PND 14 C57BL/6 mouse pups that received the surgical removal (aspiration) of the cerebellar cortex (Asp, n = 6) or the sham operation (Sham, n = 5) during manual carrying. The scale bar represents 1 mm. Immobilization times (L), body length (M), the representative posture (N), hind-limb posture (O), and tail direction (P) are shown. The surgical removal of the cerebellar cortex was verified histochemically with hematoxylin-eosin stained brain sections (Q).

In head direction, the types shown in (D) and (J) are horizontal direction (black), intermediate (dark gray), vertical direction (light gray), and shifted during the task (stripe). In the limb posture, the types shown in (E), (K), and (O) are flexion (black), intermediate (dark gray), extension (light gray), asymmetry (white), upward (hatched), and shifted during the task (stripe). In tail direction, the types shown in (F) and (P) are downward (black), intermediate (dark gray), backward (light gray), and shifted during the task (stripe). The arrows in (C), (I), and (N) indicate the body regions that exhibited significant differences between the homozygotes and the other two genotypes. *p < 0.05, **p < 0.01. All numerical data are shown as mean ± SEM. See also Figure S4.

concert, these components may be regulated by independent neural circuits in the carried animals.

To explore the underlying brain mechanism of the carrying-induced infant responses, we examined pups of several genetic mutant mouse lines exhibiting neurodevelopmental abnormalities and identified deficits in two mutants, *reeler* (*rl*) and *cerebelless* (*cbll*). The *rl* mutation results in a partial deletion in the extracellular matrix protein Reelin [18] and leads to severe hypoplasia of the cerebellar cortex. The *cbll* mutation causes a reduced expression of Ptf1a, a bHLH transcription factor, leading to a complete loss of the cerebellar cortex as well as all GABAergic neurons in the cerebellar nuclei [19]. By the second postnatal week, the general appearance and body weight of the homozygous mutant pups are indistinguishable from those of wild-type littermates (Figures S4A and S4B), and the majority of pups survive until sexual maturation despite motor coordination deficits in adults. The immobility response to manual carrying was progressively attenuated in the *cbll*/*cbll* [$F_{(2,\ 312)}$ = 13.19, p < 0.001] but not in the *rl*/*rl* [$F_{(2,\ 291)}$ = 0.34, p = 0.71] mutant pups (Figures 5A and 5G). As for postural regulation, both *rl*/*rl* and *cbll*/*cbll* pups showed overlapping abnormalities during manual carrying, including attenuated body compaction [*reeler*, $F_{(2,\ 29.42)}$ = 24.24, p < 0.001; *cerebelless*, $F_{(2,\ 20.17)}$ = 8.14, p < 0.001] (Figures 5B and 5H) and dorsoflexion of the head (Fisher's exact probability test, p < 0.001) (Figures 5C, 5D, 5I, and 5J). In addition, the *rl*/*rl* and *cbll*/*cbll* mutant pups showed the abnormal hind-limb (Figure 5E) and tail (Figure 5F) postures and forelimb (Figure 5K) posture, respectively.

Because these two genetic mutants shared a phenotype of congenital malformation of the cerebellar cortex, we further examined the role of the cerebellar cortex in the carrying-induced responses in mouse pups. PND 14 mouse pups that received surgical removal of the cerebellar cortex (Figure 5Q) showed abnormal posture regulations resembling with those of *rl*/*rl* mutant pups, namely the elongation of the body [$t_{(9)}$ = −3.411, p < 0.001] (Figures 5M and 5N), incomplete hind-limb flexion (Fisher's exact probability test, p < 0.05)

(Figure 5O), and backward tail extension (Fisher's exact probability test, $p < 0.05$) (Figure 5P) while showing an intact immobility response [$t_{(9)} = -0.966$, $p = 0.305$] (Figure 5L). These findings suggest that the characteristic compact posture during carrying is mediated by the cerebellar cortex. On the other hand, the immobility response may be mediated by the inferior olivary and pontine nuclei, which are affected only in the *cbll/cbll* mutants [19] (Figures S4C and S4D).

The Calming Response Helps Maternal Carrying

Finally, we explored the functional significance of the identified responses to maternal carrying in mouse pups. We hypothesized that if the infants did not cooperate by calming down and keeping compact posture upon carrying, the maternal burden of carrying should increase. As we have shown in Figures 3C and 3D, the proprioceptive dysfunction by pyridoxine treatment inhibited the immobilization response during carrying. We found that the time required for maternal rescue (from the time when the mother picked up the pup to the time when the mother and pup got out of the cup) of the pyridoxine-treated pups was significantly longer than that of the saline-treated pups [$t_{(16)} = 2.33$; $p < 0.05$] (Figure 3E), suggesting the importance of immobilization for maternal carrying. Next, to test the role of pups' postural regulation for maternal carrying, pups received general anesthesia prior to the maternal rescue task. This intervention made the pups completely limp and immobile, with all of their limbs extended (Figure 3F). Again, the maternal rescue time of the anesthetized pups was significantly longer than that of the saline-treated pups [$F_{(3,40)} = 15.48$, $p < 0.001$] (Figure 3G). It should be noted that the mothers rescued all of the pups irrespective of the treatments, indicating that the maternal motivation to rescue the pups was not hindered by the treatment. These data collectively indicated that both aspects of carrying-induced responses of immobilization and postural regulation contributed for facilitating the maternal carrying.

Discussion

In a variety of mammalian species such as cats, lions, rats, and galagos, it has been acknowledged that maternal oral transport induces a passive and compact posture with hind legs drawn up in carried infants [20–22]. This postural regulation has been studied experimentally in laboratory rats as "transport response" [10, 11]. However, no quantitative measurements for immobilization during carrying, investigation of physiological aspects of the phenomenon, or search for comparative nature of this phenomenon in mammalian species have been performed. This study is the first to establish the striking similarities of the carrying-induced calm state between human infants and mouse pups as an orchestration of reduced mobility, distress vocalizations, and heart rate.

In the mouse model, both the tactile sensation from the skin at the maternal grasp and proprioception were required to elicit the *carrying*-induced calming responses. This finding is consistent with previous literature reporting that maternal touch and rhythmic rocking (vestibular-proprioceptive stimulation) is calming to human infants [15, 16]. However, the effect of rocking on behavioral and physiological calming was variable among studies [23, 24]. Maternal walking may be the most ethologically relevant stimulation and provides infants with calming sensory inputs in a synergic manner, which may be more effective in calming infants than other kinds of rhythmic motion such as mechanical rocking.

Furthermore, the immobilization and the adoption of a compact posture facilitate maternal carrying, as shown in Figures 3E and 3G. Therefore, the calming responses may increase the survival probability of the infant in cases of emergency escape by the mother-infant dyad and ultimately work to support the mother-infant relationship. Conservation of this calming response in altricial mammalian species supports the adaptive value of this behavior in mother-infant relationship and, as a consequence, infant survival [10, 20, 22]. Interestingly, Vrugt and Pederson found that the effectiveness of rocking in calming infants was largest at the highest rocking frequency tested (1.5 Hz) [16], further suggesting the importance of the calming response especially during fast maternal walking in an emergency. However, in the present experimental design, we did not control for nor measure the actual maternal walking speed. Future studies with precise measurements of maternal walking speed will be required to accurately characterize its effect on the calming of infants. This calming response to maternal transport may develop even before birth in humans; at 36–40 weeks of gestation, the fetuses are more active when the mother is not active within a day [25]. Together with current findings and these previous results, we propose that carrying-induced calming represents a canonical set of behavioral and physiological responses in altricial mammalian infants and functions to facilitate an efficient mother-infant relationship.

The present study provides immediate implications for general parenting practices. The identified effects of *carrying* on parasympathetic activation and cry reduction were significant and robust, so that a brief period of *carrying* could be an effective approach to soothe crying caused by transient irritations such as vaccinations or frightening noises. However, because the calming effect was limited to the period of actual maternal walking, the infant could resume crying if the underlying cause remained after the end of *carrying*, like hunger or chronic pain. A scientific understanding of this physiological infant response could prevent parents from overreacting to infant crying. Such understanding would be beneficial to parents by reducing frustration, because unsoothable crying is a major risk factor for child abuse [26]. Additionally, our simple carrying assay of human infants might be utilized in evaluation of the autonomic functions and sensory integrations of neurological disorders in early infancy, such as autism spectrum disorders (ASDs): infants with ASDs are reported to have difficulties in cooperative adjustment of their body to parental holding [27]. Moreover, abnormalities of cerebellar structure [28] and in sensory integration [29] are among the most consistent neuropathological findings in ASDs. This study also provides implication for neuroscience research field. Most obviously, in any subsequent studies involving experimental handling of preweaning rodent pups, the carrying-induced physiological responses should be acknowledged to avoid unexpected autonomic influences on the subsequent measurements that are influenced by the autonomic nervous system, such as heart rate. And, more importantly, these results may contribute to elucidation of neurobiological mechanisms governing social bonding and cooperation across mammals. We anticipate that the identified mouse behavioral model will be a powerful tool for investigation of underlying neural mechanisms of infant contribution to maternal carrying and its dysfunction in early infancy.

Experimental Procedures

The study was approved by the Ethical Committee of the University of Trento and the Ethical Committee of RIKEN. The ECG of human infants was monitored with a SEER Light WP Holter ECG recorder (GE Healthcare) with two channels and was analyzed with the MARS8000 holter analysis system (GE Healthcare). The mouse ECG was recorded with an amplifier (UAS-308S, Unique Medical), an electrocardiogram (ATC-402, Unique Medical), and two custom-made electrodes (0.7 mm diameter, Unique Medical) placed on the proximal part of the right and left forelimbs, and then analyzed using the Unique Acquisition (Unique Medical) software to identify and label each QRS complex. Video analyses of voluntary movements were performed by at least two raters who were blind to the experimental manipulations. Statistical analyses were performed with a configural frequency analysis, Friedman test, Fisher's exact probability test, general linear model, Welch's ANOVA, and Welch's t test, where appropriate, with significance set at $p < 0.05$ after a p value correction with Holm's method. All of the statistical analyses were conducted with R 2.9.0 (R Project for Statistical Computing open source software, http://www.r-project.org/). Other experimental procedures and associated references are available in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes four figures, three tables, Supplemental Experimental Procedures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.03.041.

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