Title

Infant attachment behaviors reflect the parenting style of individual caregiver in common marmosets

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Abstract

Children's physical, cognitive, and emotional maturation require adequate interactions and secure attachment with their primary caregivers. However, the causal relationships between parenting components and the aspects of infant attachment behaviors toward the caregiver remain unclear. New World monkey common marmosets provide an ideal model of human parent-infant relations because, like humans, infant care is shared among family members using intricate vocal communications. Combining the natural variations in parenting styles and subsecond-scale analyses of dyadic vocal and physical interactions, we demonstrate that marmoset infants signal their need by the social context-dependent use of call types and by exhibiting physical avoidance to rejective and neglectful caregivers. When deprived of an early social environment, infants cannot develop the adaptive use of attachment behaviors or age-appropriate autonomy. These data demonstrate the critical role of social contexts and developmental dynamics in physical and vocal attachment behaviors in infancy.

Teaser

Marmoset infants modulate their attachment behaviors toward each caregiver, according to their inherent parenting styles.

MAIN TEXT

Introduction

Early life adversity affects infant cognitive, social, and emotional development, ultimately increasing the risks of various medical conditions and premature death [1-4]. Among the factors comprising the early life environment, the stability and quality of the relationship with the primary caregiver are critical for infants' sense of security because infants are born immature and require extensive care for survival among all mammals. The experiences gained through interactions with the primary caregiver(s) (often the mother and other family members) are also essential for learning life skills and social behaviors in many species. Thus, infants have an innate motivation to seek and maintain proximity with the primary caregiver by a physical approach and signaling their need, collectively called the attachment system [5].

While the basic attachment system is innate, infants adjust their attachment pattern depending on the quantity and quality of care received. In humans, an appropriate caregiver is supposed to function as a *safe haven*, to which the infant returns for comfort and support, and as a *secure base*, from which the infant restarts exploration in the environment with a sense of security [6]. It is theorized that if the caregiver inflicts fear or is insensitive to infant distress, infant attachment becomes "insecure", i.e., infants are not fully confident in the caregivers' availability and responsiveness [7-10]. However, the discerned association between quality of rearing and attachment security is not large, possibly due to difficulties in controlling for other parameters such as genetic factors and the role of multiple caregivers in human studies [11-14]. Thus, to further dissect the developmental mechanisms of infant attachment security, a nonhuman animal model should be established [15]. Rodents have been studied extensively [2,16-19], but the attachment behaviors of rodent pups are less selective to a particular individual, and pups do not reject other mothers. Moreover, isolation rearing only mildly impacts future social behaviors in rodents [20-22]. In Old World monkeys, maternal separation or deprivation has been established to affect infant physiology, stress responses, cognition, and later social behaviors [23-26], although direct examinations of infant attachment security

have been limited (see [27,28]). Moreover, the mother is the sole primary caregiver in Old World monkeys, unlike humans [8,29,30].

Biparental or cooperative infant care in primates is limited to several family-living species, including New World monkeys Callitrichidae (marmosets and tamarins), *Plecturocebus* (titi monkeys), and *Aotus* (owl monkeys) [31-36,37 ,38]. Among these, common marmosets (*Callithrix jacchus*) are a promising primate model with cutting-edge research resources [39-43]. At one birth, two infants are generally raised and carried almost continuously during the first postnatal months. Infant carrying impedes the carrier's locomotor activities and thus is shared by the family members: the mother, father, and older siblings [44]. Our previous study [45] identified two independent (allo)parenting parameters, *sensitivity* to infant distress and *tolerance* to infant carrying, similar to parenting styles established in humans [46-48]. Furthermore, the molecularly defined subregion of the medial preoptic area in the basal forebrain specifically regulates caregivers' *tolerance* in marmosets [45]. These data suggest the common neurobiological mechanism of infant caregiving behaviors across primates.

Additionally, marmosets' vocal development has attracted considerable attention because some of its characteristics are similar to those of human language, e.g., turn-taking and babbling [39,49]. Undirected (isolated) vocalizations of infant marmosets undergo progressive changes from infant-specific "cry" and babbling to tonal "phee" calls. These changes have been attributed to the maturation of the vocal apparatus [50,51] and social learning from vocal feedback from parents [52-54]. Nevertheless, as very young infants are continuously carried by the caregiver and infant calls function to attract parental approach [55,56], an investigation of infant call development within the context of parent-infant relations shall provide new insight into vocal learning in marmosets [57-59].

Thus, this study investigates the relationship between parenting styles and infant attachment behaviors, including vocal communications, utilizing the high natural variations of the parenting parameters and the experimental manipulation of rearing conditions.

Results

The infant retrieval assay to study caregiver-infant interactions

The families were kept in a large family cage consisting of two to three connected cubicle cages. The infant retrieval assays, or "brief separation and reunion" from the infants' viewpoint, were conducted utilizing two cubicle cages within their home cage. A caregiver, either a mother, father or an older sibling of the infant, was placed in one cage, and an infant in a wire basket was placed in an adjacent cage connected via a tunnel with a shutter (Fig. 1a) (Supplementary movie 1). After the shutter was opened, the caregiver typically entered the infant cage, approached, leaned into the basket, and came in contact with the infant. The infant climbed over the caregiver’s trunk, designated as infant retrieval and the start of carrying (Fig. 1b). After retrieval, the caregiver carried the infant for varying durations and may have eventually started rejecting the infant by rolling on the floor, pushing, and biting the infant. These behaviors often caused the removal of the infant from the carrier's body (Supplementary movie 2). The session was continued for 600 sec after the first retrieval or from the assay start (see Table S1, Methods).

Detailed analyses of 286 infant retrieval assay sessions were conducted involving 7 families, 25 infants, and 55 infant-caregiver dyads from postnatal day (PND) 1 to 36 (Table S1 and

Fig. S1). The caregiver's and infant's behaviors and calls listed in Table S2 were analyzed at a subsecond scale, with 0.2-sec bins using video and vocal recordings of the sessions. Based on the caregiver-infant interactions, the total period of each assay was broken down into five social contexts, which were mutually exclusive and collectively exhaustive (Fig. 1b): *Alone_BeforeRET,* when the infant was not carried yet before the first retrieval; *Holding,* when the caregiver was carrying the infant without locomotion; *Transport,* when the caregiver was carrying the infant and locomoting; *During_Rejection,* the period from the start of rejection to 9.4 seconds after the end of rejection (see below and Fig. 1b legend for this definition); and *Alone_AfterRET,* when the infant was not carried after the first retrieval occurred (Table S2).

Caregiving parameters define parenting styles

We first examined the correlation matrix of all the parameters of caregivers' and infants' behaviors (Table S2) for each infant retrieval assay (the left-bottom half of Fig. 1c) or those parameters averaged for each caregiver-infant dyad (the right-top half) during postnatal weeks 0-4. We also utilized the parameters representing the dyadic relations in other social contexts, such as undisturbed family observations and food-sharing experiments obtained in our previous study [45]. From this matrix, the significant correlations between parameters were screened and further analyzed.

Here, we defined three caregivers' parameters: (i) the <u>retrieval latency</u> (*RET_Latency*), the time required for infant retrieval, which negatively represented the *sensitivity* of the caregiver toward infant distress vocalizations; (ii) the <u>rejection rate</u> (*%Rejection*), which negatively represented the *tolerance* of the caregiver to infant carrying; and (iii) the <u>carrying rate</u> (*%Carry*), the total carrying duration divided by the session length, which represented the total infant care quantity. *RET_Latency* and *%Rejection* were mutually independent ($r = 0.2338$, $p > 0.05$), and both contributed to net infant carrying care (*%Carry – RET_Latency*: $r = -0.5946$, $p < 0.001$, *%Carry - %Rejection*: $r = -0.5969$, $p < 0.001$). These caregiving parameters were highly correlated with the caregiving parameters in family settings (e.g., *FamilyRejectionRate(B)*) [45], indicating the existence of a caregiver-inherent "(allo)parenting style".

Along with infant development, the latencies of caregivers' approach to the infant increased (Fig. 1d), while *%Rejection* remained consistent (Fig. 1e). The net *%Carry* (Fig. 1f; $r = -0.2845$, $p = 0.0067$) and the caregiver-infant contact declined after postnatal week 4 ($r = -0.2808$, $p = 0.009$), indicating that the parent-infant interactions were essentially stable during the first postnatal week. No significant differences among mothers, fathers, or older siblings were found in these caregiving parameters (Fig. 1e, f).

"Approach" components of the attachment system of infant marmosets

Infants seek and maintain the proximity of specific familiar individuals using two kinds of attachment behaviors: 1) *approach* (seeking, following, clinging) and 2) *signaling* (crying, smiling and gestures) [5]. In the first postnatal month of marmoset infants, "approach" behaviors were manifested mainly by actively clinging to the caregiver's body whenever the caregiver made contact and by not breaking the contact unless the caregiver rejected them. *Infant_Moving_Alone* or spontaneous locomotor activities were positively correlated with infant age ($r = 0.2979$, $p = 0.0022$) (Table S2) (Fig. 1c).

To examine the *selectivity of infant approach* behavior, we compared the speed of infants clinging to their parents or unfamiliar adults with multiple parental experiences (Fig. 2a). Infants instantaneously clung to their parents but not to the unfamiliar caregivers (Fig. 2a, left). In contrast, parental latencies to contact with their own or unfamiliar infants were highly variable and did not reach statistical significance (Fig. 2a, middle, the generalized linear mixed model (GLMM), $p = 0.2040$). The carrying rate, which both infants and parents could contribute to, was low for the unfamiliar parent-infant dyad (Fig. 2a, right). This selective attachment toward their own parents was attributed to familiarity rather than biological relatedness because fostering early neonates is generally successful in marmosets [60]. Taken together, these results strongly suggest that marmoset infants develop a selective attachment with their family caregivers.

Contact-breaking behavior by infants was seen in two ways: one was when the infants were in contact with a caregiver but did not cling (passive), and the other was voluntary dismounting from the caregiver's body (active). Practically, however, it was not straightforward to determine whether an infant's dismounting was forced by caregivers' rejection or by infants' voluntary action. To empirically determine the direct influence of the preceding rejection on infant dismounting, we performed a segmented regression analysis to identify an abrupt change in the response function of a varying influential factor [61]. The result in Fig. 2b identified the inflection point at 9.4 sec; thus, we defined *During_Rejection* as the period from the onset of rejection to 9.4 seconds after the end of rejection (Fig. 1b). The dismounting that occurred after a 9.4-second offset or those without preceding rejection were regarded as voluntary.

An infant's *avoidant behaviors* were defined as the sum of voluntary dismounting and absence of clinging when the infant was in contact with the caregiver. The frequency of avoidant behaviors per session was substantially increased after postnatal week 4 (Fig. 2c), indicating that the marmosets become autonomous. Thus, these results suggest that avoidant behaviors after postnatal week 4 are a sign of typical development, while premature (i.e., within postnatal weeks 0-3) avoidant behaviors are atypical and may be a sign of decreased infant attachment to the given caregiver (see below).

Infant call as a "signaling" component of the marmoset attachment system

Calls during rejection. Next, we studied infant vocalizations in various social contexts with the caregiver. The infants emitted calls most frequently when they were rejected or immediately after (Fig. 2d, *During_Rejection;* Supplementary movie 2, Fig. S2a). In the family observation, the infant's vigorous calls during rejection appeared to attract other family members to the infant-carrier dyad and allow the infant to transfer quickly from the previous carrier to the next (Supplementary movie 3), suggesting the signaling function of infant calls. In this way, during the first three postnatal weeks, most infants directly transferred from one carrier to the next (see below Fig. 5c).

Calls while not being carried. Infants also called frequently when not carried (Fig. 2d), often as a continuous string of various different call types termed "babbling" (Fig. 2e) [49,57]. We observed that these intense bouts of calling stopped immediately after the infants were carried (Fig. 2d-e). Detailed comparisons of call frequencies at the transition of social contexts revealed that infants reduced calling when they came into contact with the caregiver with any part of the body and further withheld calling when they clung to the caregiver's body (= carrying) (Fig. 2f). These findings further support the notion that these bursts of infant calls in *Alone* contexts signaled the separation

distress widely observed in mammalian infants [62] and thus were withheld immediately after contact with the caregiver.

Ontogeny of calls in family and isolation. The call frequencies during each social context did not change until postnatal week 5 (Fig. 2g). The increase in total calls during the test session along with infant development (Fig. 2g, right) should be attributed to the significant increase in *Alone* contexts (Fig. 1g). In a separate experiment, when we briefly isolated these infants and recorded their vocalizations in isolation, total call frequencies were high in the beginning and declined after postnatal week 6 (Fig. 2i), when parental carrying declined rapidly [45]. These findings altogether suggest that the calls during *Alone* contexts (calls while not being carried in the family or in isolation) decline during infant development due to the decline in attachment needs by infant maturation (see Discussion).

Selective use of call types. Infant marmosets emit various kinds of call types, including twitter, tsik, trill, phee, and vhee [also termed "ngâ" [63] or "cry" [53,64]; here we use vhee to avoid unintended anthropomorphic interpretation] calls as well as various combinations of these calls (Fig. 2e, i, S2b, c). The ratio of infant call types depended considerably on the social context; infants emitted more trill calls when they were carried and more tsik calls when they were rejected (Fig. 2h). Twitter and vhee calls were most frequent during *Alone_BeforeRET* and *Alone_AfterRET*, respectively. These call type usages did not change substantially during postnatal weeks 0-5 in the infant retrieval assays (Fig. S2c). Thus, infant marmosets in the first postnatal month already use multiple call types selectively in each social context, although not exclusively.

In the isolated recording condition, while the total amount of phee calls remained stable across development (Fig. 2i, Fig. S3b), vhee and twitter calls rapidly declined during the first 8 weeks (Fig. 2i, Fig. S3c, f), which accounted for the decrease in total calls, indicating that the developmental increase of phee calls was relative and caused mainly by the developmental decline of total calls, especially vhee and twitter calls in isolated recording conditions.

Infants tune their attachment behaviors according to the parenting styles of each caregiver

We next investigated the relationship between parenting styles and infant behaviors by utilizing the wide range of individual variabilities in caregivers' parameters (Fig. 3a-c). As an example, male twin infants Saku and Gaku (Fig. 3a) had a tolerant mother, Kachan, and an intolerant, rejective father, Tochan, which occasionally attacked his infants. In their representative retrieval sessions, the mother (Fig. 3a, top panels) retrieved the infant quickly and carried it throughout without rejections. Both infants called only during *Alone_BeforeRET,* except for occasional trill and phee calls by Saku. The infants did not show any avoidant behaviors toward their mother, typical for this age. In contrast, the father (Fig. 3a, bottom panels) repeated retrieval and subsequent rejection, resulting in fragmented and short carrying bouts. In the father's sessions, both infants exhibited premature avoidant behaviors (black triangles in Fig. 3a) not only during *During_Rejection* but also during *Carrying* (*Holding* + *Transport*). Moreover, the infants did not immediately withhold calling after the father's retrieval (for example, approximately 600 sec in the left-bottom panel). These patterns of parental behaviors were consistent across litters (Fig. 3a, left and right), throughout infant development and across two births (Fig. 3b left, dark blue for Tochan, piriangles for Kachan). These observations suggest that each parent behaved similarly toward different infants, and each infant behaved according to the caregiver's behavior.

Avoidance and anxious calls during carrying are associated with a low quantity and quality of caregiving

The above observations suggest that there are at least two kinds of atypical attachment behaviors of marmoset infants toward inappropriate caregiving: i) avoidant behavior, namely, dismounting and refusal to cling at contact, exhibited prematurely (i.e., within postnatal weeks 0-3) and voluntarily (i.e., not in *During_Rejection*); and ii) calls while being carried, namely, infant calls during the caregiver's carrying. Indeed, the mappings of individual dyads for the average caregiving parameters (Fig. 3b, left) and the average atypical attachment behaviors (Fig. 3b, right) impressively resembled each other. The atypical attachment behaviors were much more frequent with the caregivers showing either *%Rejection* or *RET_Latency* over the mean + 1 S.D. (Fig. 3c, bottom) than the rest of the caregivers (Fig. 3c, top).

To statistically examine the correlations between parenting styles and infant attachment, we divided dyads into two groups, high/low groups for avoidant behavior and calls while being carried, and compared three caregivers' parameters between the high/low groups using the generalized linear mixed model (GLMM). The amount of infant avoidant behaviors was correlated with all the caregiving parameters *RET_Latency*, *%Rejection*, and *%Carry* (Fig. 4a-c, Fig. S2d), suggesting that infants avoid any caregivers showing insensitivity, intolerance or scarce carrying. On the other hand, the amount of calls while being carried was associated with *%Rejection, %Carry* but not with *RET_Latency* (Fig. 4d-f, Fig. S2d), suggesting that infants call more while being carried by rejective caregivers but not by insensitive caregivers. The call type-specific analysis revealed that during carrying by rejective caregivers, negative calls (tsik and vhee) were emitted more frequently, and the positive trill call was emitted less (Fig. 4g). Considering the fact that tsik and vhee calls were frequently emitted during isolation, and trill calls were emitted while being carried (Fig. 2h), infants might be insecure and exhibit anxiety-like call when they were carried by rejective caregivers. These atypical attachment behaviors were positively correlated with each other (Fig. 4h).

These correlations, however, do not infer the causality between caregivers' and infants' behaviors, and it is still possible that the atypical infant behaviors affect caregiving behaviors. We next compared the variabilities of atypical attachment behaviors within the same infant toward different caregivers (SI-DC, gray segments in Fig. 4i) vs. variabilities within the different infants toward the same caregiver (DI-SC, colored lines). We found that the variability between different caregivers and the same infant was larger (Fig. 4j). Together with our previous analysis showing the inherent nature of sensitivity and tolerance in each caregiver [45], this result suggests that the caregiver-infant relationship is determined more by consistent parenting styles of each caregiver than by infant predispositions.

Family-separated, artificially reared infants as a model of highly insensitive caregiving: Study design and an example

The above-described data strongly suggest that parenting styles causally determine the pattern of infant attachment behaviors. Nevertheless, as these data are observational, the findings should be confirmed by interventional experiments (see [26]). To this end, we utilized marmoset individuals who were separated from their families in infancy and reared artificially, modeling extremely insensitive caregiving. We collected 5 artificially reared infants (Art) from our breeding colony, which were born as triplets or to a mother with dysfunction of one nipple (detailed in Table S6) and could not be maintained in the family even with supplemental formula feeding. Six of their

littermates or age- and sex-matched infants were used as controls (Cont). These Art infants were housed individually and reunited with the original family at least 3 hours per day for 3-7 days per week in the daytime, except for one (Michael), which was rejected from the family in several trials of reunion and was once attacked (Table S6). The subjects were followed from birth and examined along with their development with family reunion observations and infant retrieval assays (Fig. 5a).

Family reunion observations during infancy were performed with four Art and five Cont infants. The observation started by returning the infants to the home cage of their original family after body weight measurements, and the behaviors of the infants and the family members were coded in every 10-second bin. In a representative example (Fig. 5b), one Art and two Cont littermate infants at PND 31 were returned to their family cages one by one. The Art infant Senazo (the bottom row in Fig. 5b) was first retrieved by the father but soon dismounted because of the father's rejection (dark blue bins). The experimenter assisted Senazo in clinging to the mother, which was tolerant and was carrying two Cont infants (Yoshie and Shirizo, the top and middle row of Fig. 5b). The mother carried three infants (10th to 20th pink bins) and then rejected them (21st dark purple bin). Senazo dismounted, while two Cont littermates kept clinging even though the mother kept rejecting them for 6 consecutive bins. In the meantime, Senazo came into contact with the brother (24th-26th light green bins) but did not cling to the brother (retrieval refused by the infant, orange mark above the bin). Senazo called in two long bouts from the 29th and 46th bins (yellow marks), came into contact with the brother and the father sequentially but refused to cling. The two Cont infants did not call while being alone after dismounted from the father at the 34th bin, which showed age-appropriate maturity (Fig. 2g). In contrast, Senazo showed excessive distress calls (which should attract caregivers) but avoided simultaneously the behavior reminiscent of "disorganized" attachment in humans. In addition, compared to Cont littermates, Senazo tended to give up clinging and dismount easily by brief rejection.

Artificially reared infants showed avoidance and age-disproportionate distress in family settings, even though the caregivers accepted them

The differences in the parent-infant relationship between Cont and Art infants during the family reunion observation were statistically examined in the GLMM, with explanatory variables of Art/Cont and PND. Typically, when an infant was returned to the home cage after a transient removal (e.g., for body weight examination), the caregivers often approached the returning infants, showed aggression to the experimenter whose hand grabbed the infant, and tried to reach and draw the infant closer by the forelimb. The proportion of caregivers showing this infant approach in the family declined significantly with infant age but was not different between Art and Cont infants (Fig. 5d). Subsequent caregivers' attempts to retrieve the returned infant (caregivers' help, Fig. 5e) and caregivers' rejection (Fig. 5f) also did not differ between Art and Cont infants. Thus, marmoset caregivers appeared not to discriminate between Art and Cont infants.

On the other hand, infant avoidant behaviors toward caregivers were significantly more frequent among Art infants than among Cont infants after the second postnatal week (Fig. 5g). This might cause a decrease in the total carrying rate of Art infants, starting at a similar time (Fig. 5h).

The duration of each carrying bout, the latency for the first rejection in each carrying bout, and the total number of rejections during each carrying bout declined according to infants' development (Fig. S4a-c). Among Art infants, the total number of rejections during carrying declined faster than among Cont infants (Fig. S4c), likely due to the reduced tolerance to rejection

among Art infants (Supplementary movie 4). In concordance with this interpretation, the transfer of the infants from one caregiver to the next was different between Art and Cont infants (Fig. 5c). When rejected, Cont infants tended to stick to the caregiver until another caregiver came close and directly moved from the present caregiver to the next (direct transfer) until PND 20. However, Art infants tended to dismount to stay alone before being transferred to the next caregiver (nondirect transfer) after PND 12 (Fig. 5c). Overall, these data suggest that Art infants exhibited compromised approach components of attachment behaviors and increased avoidant behaviors toward caregivers, even though the caregivers behaved similarly toward Art and Cont infants.

Next, vocal behaviors were compared between Art and Cont infants (Fig. 5i, j). In the family reunion observations, the total calls as well as calls while not being carried were significantly more frequent among Art infants, particularly after PND 30 (Fig. 5b, i, j), indicating the age-disproportionate, excessive distress signaling of Art infants (note that in this family setting, calls while being carried could not have been assessed precisely and thus will be described below in the dyadic infant retrieval assays). We also assessed the development of vocalization of one Art infant Michael in the isolated recording condition (Fig. S3a-g). Compared with Cont infants (including Cont littermate Cubby), Michael exhibited fewer calls in the first postnatal month, possibly because Michael was used to staying alone, while Cont infants were not. After the second postnatal months, while Cont infants reduced the calls, especially vhee and tsik calls, rapidly, Michael did not show this trend, again consistent with the delayed independence compared to the family-reared infants.

Avoidant attachment in Art infants in dyadic infant retrieval assays

We next conducted dyadic retrieval assays with four Art and four Cont infants (Fig. 6a). In an example case, Cubby (Cont) and Michael (Art) were tested with their father Chuck. While Cubby was carried throughout even during and after rejection (top panel, Fig. 6b), Michael clung on but dismounted from Chuck's body soon after the first retrieval without obvious rejection from Chuck (left black triangle, Fig. 6b bottom) and then was carried again. Michael was rejected once, and a minute later, it dismounted from Chuck without preceding rejection. Chuck made contact again with Michael during 178-190 sec, but Michael did not cling onto Chuck (red triangle at approximately 190 sec, Supplementary movie 7) and kept calling. The refusal of clinging occurred again at the end of the session (red triangle, right). Overall, these patterns of atypical behaviors toward the caregiver were consistent with those observed during family reunion (Fig. 5b).

GLMM analyses revealed that the latencies of caregivers' entry into the cage containing the infant (Fig. 6c) and contact with the infant (Fig. 6d), which solely depended on caregivers' motivation, were not different between Art and Cont, while the actual retrieval latency was significantly longer for Art (Fig. 6e), possibly because of avoidance of Art infants. In the dyadic situation examined within PND 30, *%Carry* and *%Rejection* were not significantly different between Art and Cont (Fig. 6f, g). For infant behavioral parameters, infant avoidant behaviors were more frequent among Art infants (Fig. 6h), as in the family reunion observations. Infant total calls or calls during carrying did not differ between Art and Cont within PND 30 (Fig. 6i-k) (note that calls while not being carried were higher after PND 30 in family settings (Fig. 5j)). In the plot of atypical attachment behaviors for Cont and Art infants (Fig. 6m), Cont infants showed few avoidant behaviors and calls while being carried, the latter except for one dyad (Chuck-Nana; note that Chuck was inherently rejective toward any infants as seen in Fig. 3b). In contrast, Art infants often showed more avoidant behaviors (Fig. 6m). Furthermore, Cont infants clung mainly to the

caregiver's trunk, especially when they became older (Fig. 6l, top, Supplementary movie 5). However, Art infants often clung onto the head or neck (Fig. 6l bottom, Supplementary movie 6). In summary, Art infants showed multiple atypical attachment behaviors, even when caregivers treated them equally to Cont infants in both family reunion and dyadic situations, indicating the pivotal role of social contact during the first two postnatal weeks in the proper development of the infant attachment system.

Discussion

Parenting styles in primate caregivers

This study investigated caregiver-infant interactions in common marmosets with high temporal resolutions and first elaborated on caregiver-inherent (allo)parenting styles, comprising sensitivity, tolerance and caregiving quantity operationally defined in dyadic interactions. These parenting parameters showed significant consistency within each caregiver across infants and experimental paradigms. Moreover, the sensitivity-tolerance dimensions of each caregiver had substantial agreement with studies on maternal styles in Old World monkeys [65-70] and humans [5,8,46-48,71]. Of course, human psychological constructs are much more complex than behavioral parameters in marmosets. Nevertheless, it is an intriguing consistency concerning the evolution of primate parenting and deserves further investigation.

Typical and atypical infant attachment behaviors in response to individual parenting styles

This study established that isolated marmoset infants call vigorously, and upon contact with the familiar caregiver, they immediately cling on and withhold calls. This clearcut on-off regulation of the infant vocalizations may solicit caregivers' reinforcement learning to retrieve the infant, as loud infant calls should attract predators in the natural habitat. Both signaling and approach behaviors decline after one month of age, when the infants gain their locomotor independence. All these features are in concordance with the core concept of the infant attachment system as defined by Bowlby [5].

Family-reared infants are shown to tune their attachment behaviors flexibly toward each caregiver, according to the natural variation of caregiver-inherent (allo)parenting styles. Both rejective and insensitive parenting increase premature avoidant behaviors in infants, even when other carriers are unavailable. These avoidant behaviors may be interpreted as the diminished *safe haven* quality of the caregiver-infant interaction. Another atypical attachment identified here is the insufficient turning-off of calls upon carrying by intolerant caregivers. Infants still cling to the intolerant caregiver but simultaneously emit more negative calls (tsik and vhee calls) than trill calls (Fig. 4g), as if they were still alone. Calls while being carried may thus indicate anxiety and a decreased sense of security. As the sense of security obtained in the proximity of the caregiver should be a prerequisite for the subsequent redeparture from the caregiver, calls while being carried may also be relevant to hindered *secure base* behavior.

It should be stressed here that these apparently "insecure" attachment behaviors of family-reared marmoset infants are not a fixed "style" or predisposition because infants selectively show secure or insecure attachment toward each caregiver (Fig. 3a). This finding points to the advantage of studying marmosets, in which infant attachment is shared among multiple caregivers, as in

449 humans. While infants of macaque monkeys, dogs, and chicks have been reported to show unaltered
450 or even stronger clinging when their attachment figures behave abusively (see [72]), marmoset infants
451 show evident avoidance in this study, possibly because they can still rely on other caregivers.
452 Marmoset infants may be more resilient when one caregiver is abusive, while macaque infants
453 receive direct effects from abusive mothers and show delayed independence and excessive anxiety
454 [27].
455
456 **The effects of artificial rearing in marmoset infants and comparisons with disorganized**
457 **attachment and other attachment-related issues in humans**
458 In contrast to the flexibility of the attachment system in family-reared marmosets,
459 artificially reared infants show rigid, fixated avoidance of any caregivers. Moreover, they do not
460 develop age-appropriate autonomy and call excessively while not being carried after one month of
461 age. These calls of Art infants do not function to gain proximity to the caregiver because they
462 simultaneously show contact avoidance. These paradoxical behaviors of the Art marmosets
463 resemble the disorganized attachment [73] or the inhibited type of reactive attachment disorder [74]
464 documented in humans. This study is too preliminary to propose these behaviors of Art marmoset
465 infants as a primate model of these attachment disturbances in humans. Nevertheless, the present
466 results highlight the pivotal role played by the caregiving experienced in the first postnatal month
467 (approximately the first year for human infants) in shaping both approach and signaling components
468 of attachment behaviors, thus forming a basis for dissecting neuromolecular mechanisms of infant
469 attachment and its dysregulations. We are currently studying whether and how Art infants' atypical
470 social and nonsocial behaviors extend into adulthood and which brain areas are involved in
471 separation distress and reunion behaviors in young marmosets.
472
473 **Vocal development and the attachment system in marmoset infants**
474 This study shows that the developmental shift from vhee to phee calls in isolated conditions
475 is mainly caused by the rapid decrease of vhee and twitter calls in the first two months, resulting in
476 the relative increase of phee calls, while the absolute amount of phee calls is stable during the
477 postnatal 3 months (Fig. 2i, S3). Moreover, our analyses demonstrate that infant vocalizations are
478 highly dependent on social contexts rather than on infant age during the first 6 weeks (Fig. 2g, h,
479 S2c). These findings collectively suggest that the developmental decrease in vhee calls and the
480 relative increase in phee calls under isolation can be at least partly caused by the developmental
481 decrease in infant need for carrying. This notion is supported by the previous finding that even
482 young adults emit vhee-like calls in a certain context, such as subordination [63]. It is possible that
483 vhee (cry) calls can be used for begging-like social intent of marmosets in general, and such
484 situations decrease with infant maturation.
485 Chronic inhibition of naturalistic parent–infant interactions disturbs both early vocal
486 learning and infant attachment formation; the latter hinders the maturation of autonomy and affects
487 regulation in primates. Both vocal learning and attachment formation can independently influence
488 vocal patterns, depending on social contexts and developmental stages (e.g., Fig. S3a-g). As a result,
489 these data are complex but are coherently understood utilizing the concept of the infant attachment
490 system, which requires proper parental care to be matured [23,26].
491 We quickly add that the present findings neither contradict nor disregard the importance
492 of vocal learning from adult feedback. We also admit that our study is not elaborated or sufficient

for vocal analyses, as our main focus is on parent-infant relations. The present study instead proposes the infant attachment system as an *additional* regulator of infant vocalization in various social contexts. Appreciating infant vocalization as a signaling component of the attachment system may help a precise understanding of the development of vocal patterns in infant marmosets, including babbling.

Materials and Methods

Animals (normative family)

All animal experimentation was approved by the Animal Experiment Judging Committee of RIKEN and was conducted following [75]. Common marmosets were reared at the RIKEN Center for Brain Science in accordance with the institutional guidelines and under veterinarians' supervision.

We tested 65 common marmosets from 9 families: 8 fathers, 9 mothers, 32 siblings (19 males and 13 females, 3 out of 32 were also included as two fathers and a mother later), and 49 infants (29 males and 20 females, 30 out of 49 were also included as siblings and/or fathers later). Detailed information about the subjects is shown in [45]. They were housed as a family, ranging from parents and two infants (minimum) to parents, two older siblings, two younger siblings, and two infants (maximum). The average number of infants at one birth was 2.21. When triplets or quadruplets were born, the extra infant(s) were used for artificial rearing experiments or removed from the family at PND 0-2 to be subjected to histological experiments. In Chuck's family, the mother (Fastener) was able to nurture only one infant at a time because of the dysfunction of one nipple. Thus, another infant was removed or, in one case, artificially reared (see below).

One cage was 43 (width) × 66 (height) × 60 (depth) cm. Two or three cages were joined through a square hole (9.6 cm wide × 10.5 cm high) on a side panel or a metal mesh tunnel (75 cm wide × 30.5 cm high × 21 cm deep) placed in front of two cages, depending on the number of family members in accordance with the ethical guideline of RIKEN, to form one single home cage. Each cage contained a food tray, a water faucet, two wooden perches, and a metal mesh loft. Although tactile contact was restricted between families, visual, olfactory, and auditory communication was possible in the colony room. Water and food were supplied ad libitum. The monkeys' food was replenished at approximately 11:30, and supplementary foods, such as a piece of a sponge cake, dried fruits, and lactobacillus preparation, were replenished at approximately 16:00. The photoperiod of the colony room was 12 L:12 D (light period: 8:00-20:00, dark period: 20:00-8:00). The observations and experiments were conducted in the animals' home cages between 8:00 and 17:00. All marmosets were well habituated to the presence of the experimenters (KS, SY, and a technical staff member) in the colony room to conduct the observations and experiments.

Evaluation of the caregiver-infant relationship

Caregiver-infant interactions were studied through four structured assays of parental behaviors as described [45]: briefly, (1) instantaneous scan sampling of the family cage, 5 times per day from PND 0 to PND 92 (minimum and maximum data points in the dataset; the same hereinafter), to record the identity of the carrier(s) and the number of infants being carried; (2) continuous 20-min focal observation of the family from PND 0 to 60, to record each family

member's caretaking behavior, social behavior, and nonsocial behavior in each 30-sec bin; (3) dyadic "infant-retrieval assay", starting from 1 to PND 41; and (4) one-to-one "food transfer assay" from PND 69 to PND 128. In our previous study, all of these data were coded on-site for caregiving behaviors and used to yield a set of parameters as summarized in Table S3 (twins are pooled because the individual was not distinguished in the observation) [45]. From this dataset, we performed microanalyses of infant retrieval assays for both caregivers and infants as described below.

Infant retrieval assay

The dyadic infant retrieval assays, or brief separation-reunion sessions from the infant's viewpoint, were based on the previous marmoset literature [45,76-78] and rodent parental behavior studies [79-81]. The stress loaded onto animals was minimized using their home cage as the testing arena, although some stress was inevitable because of the temporary separation of other family members from the testing area and a brief period (maximum 600 sec) of infant isolation.

The infant retrieval assay (Fig. 1a) was conducted from PND 1 to 41 as described [45]. An infant was presented to one of its parents or elder siblings when we returned an infant to its home cage after daily body weight measurement. If more than two caregivers from each family were tested in the same parturition or an infant was a singleton, the infants were separated twice on the same day. The order of the test of multiple family members was counterbalanced. When there were twins in one family, the stimulus infants were alternated. All family members were acclimatized to the tunnel and the wire mesh basket without infants before the test. During the test, the joined home cages were divided into three parts (43 × 66 × 60 cm each) using steel partitions. Two of these cages were connected by a mesh tunnel (75 × 30.5 × 21 cm) and used for the test (Fig. 1a). The caregiver was placed in the left cage before the start of the test. The other family members were placed in the third cage not used for the experiment. During this procedure, the caregiver and the other family members were gently separated by being lured with a piece of sponge cake to minimize the effect of handling on the caregiver's subsequent behavior. Then, the infant was gently taken up from a carrier and placed into the mesh basket (15 cm in diameter × 15 cm high), which contained a gauze-covered electric hand warmer (KIR-SE1S, Sanyo, Osaka, Japan) to maintain the body temperature of the infant during separation. The infant in the mesh basket was placed in the right cage. Opening the shutter of the caregiver's cage permitted the caregiver to access the infant's cage. The behavior of the caregivers and infants before retrieval and 600 sec after retrieval, or for 600 sec after the opening of the shutter when retrieval was not attempted, was directly observed and recorded using two video cameras (HDR-AS100V, Sony, Tokyo, Japan) as well as a directional microphone (MKH 416, Sennheiser, Hanover, Germany) connected to a linear PCM recorder (DR-60DMKII, Tascam, Tokyo, Japan). The audio was recorded at 24-bit and 96 kHz. The time from the opening of the sliding door to the retrieval of the infant, which was defined as when all of the infant's limbs were in contact with the caregiver's body, was recorded as the retrieval latency. Immediately after successful retrieval, the caregivers' infant-directed behaviors were coded on-site for 600 sec with 30-sec bins. The session ended if the caregiver did not retrieve the infant for 600 sec (or 300 sec for the initial 12.6% of these experiments). Twenty-three (2.8%) and 39 (4.8%) sessions ended without retrieval for 300 sec and 600 sec, respectively, among 815 sessions in total.

579 **Microanalysis of the infant retrieval assay**

580 Using infant retrieval assays recorded by video and a directional microphone [45], manual
581 microanalyses were completed for 286 sessions using 7 families (Table S1) and investigated in this
582 study. The video was analyzed with 0.2-sec bins for the behaviors listed in Table S2 using Solomon
583 Coder (ver. 19.08.02). Infant's and caregiver's vocalizations were detected and classified from the
584 spectrogram of the audio using PRAAT (ver. 6.0.43). Out of 286 sessions, we excluded 115 sessions
585 without high-quality vocal recordings from the detailed vocal analyses.

586 In the statistical analyses, 21 sessions during the first postnatal week with the caregiver
587 who performed the infant retrieval assay for the first time were regarded as training and excluded
588 from the statistical analysis [45]. The percentage of rejection (*%Rejection*) and infant calls during
589 carrying were calculated in a session with at least one retrieval. The dyadic averages of these
590 parameters were not calculated in an infant-caregiver dyad, Pearl-Mogol, because Mogol did never
591 retrieve Pearl in the three sessions used in the analysis.

592 Among 164 observed dismounts, 34 dismounts occurred without a preceding rejection.
593 The other 130 dismounts occurred after the rejection with variable intervals ranging from 0.4 sec
594 to 417.2 sec. To divide these events into forced and voluntary dismounts, we performed log-
595 survivorship analysis for rejection-dismount intervals and found that the inflection point was 9.37
596 sec. The rejection-dismount intervals shorter than this threshold were regarded as influenced by the
597 preceding rejection (66.5%, 109 out of 164 dismounts). Twenty-one dismounts with an interval less
598 than the threshold and 34 dismounts without any preceding rejection were regarded as voluntary
599 dismounts (33.5%).

600 To examine the selectivity of infant attachment, we conducted an additional seven sessions
601 using three infants and two unfamiliar, biologically unrelated adults (one male, Oji, and one female,
602 Hime) with multiple parental experiences. The unfamiliar adults were employed for the experiment
603 when they did not nurture preweaning infants. To minimize the chance of aggressive attacks toward
604 the infants by unfamiliar adults, we selected the adults with comparatively less rejective as
605 caregivers and monitored their behavior on-site to be able to interrupt the experiments if the adults
606 attacked the infants. In the present study, there was no aggression by the adults exceeding the level
607 of normal rejections. To evaluate the caregivers' behavior, seven sessions with two of their own
608 infants were compared as controls (own infant). For the control of the infants' behavior (own
609 parent), seven sessions with two mothers and two fathers of the infants were used. The sex of
610 caregivers and age of infants in the control sessions were matched.

611

612 **Artificially reared infants**

613 In our breeding colony, we could often maintain the whole litter of triplets with
614 supplemental feeding but not always, depending on the capacity of milk production of the mother
615 and the total caregiving motivation of the family members. We utilized 5 infants, each out of such
616 triplet cases (or a twin in the case of Chuck's family, as shown above) The detailed rearing
617 conditions are described for individual cases in Supplemental Table 6. Briefly, infants were isolated
618 from their families on the day of birth and fed milk and weaning food, chow soaked in milk and
619 human baby food until weaning. They were kept individually in small cages (e.g., 22 × 14 × 14 cm)
620 placed in an incubator to control the temperature (30–37°C). The cage size and the temperature
621 were changed according to their growth. Although tactile contact was restricted between other
622 marmosets, including their families, visual, olfactory, and auditory communication was possible in

the colony room. Michael was continuously used for vocal recording in the insolated condition (see below) after weaning. After weaning, Michael was put into a normal home cage (43 (wide) × 66 (high) × 60 (deep) cm) with normal chow and water (ad libitum) and singly housed to avoid aggression from other individuals. Marmosets reared by their family were used as control subjects. In the family-reunion observations and the retrieval assays, the littermates of the artificially reared subjects were used as control subjects, except for Atako. Atako was artificially reared because it was a triplet born in a family that was in another experiment. To prevent disturbing the original family, all of Atako's experiments were conducted in the Chuck family, which bore two infants one day before Atako's birth. Therefore, Atako had a control outside of the litter.

Family reunion observations of artificially reared infants

Four Art and five Cont infants (Table S7) were repeatedly employed for the family reunion observations during PND 1–53. The observations were performed once to three times per week. The observations were conducted in a home cage (size is described in Table S7) that contained a mother, a father, and one to three older siblings. Family-reared infants were temporarily removed from the home cage and then artificially reared, and family-reared infants were introduced into the cage in random order. The length of stay in the home cage with observation varied between 16 and 119 min (mean ± standard deviation: 57.97 ± 25.93 min).

The caregivers' reaction when the infant was returned to their home cage was categorized as follows: approached, the caregiver approached the cage entrance where the infant was released by the observer; and helped, the caregiver helped the infant to hold its body or tried to attack the observer's hand holding the infant.

The following infants' conditions and behaviors were manually categorized using *one-zero sampling* in 10 sec bins: condition - carried, rejected, attacked, and touched by family members; behaviors - calling. The infants' calling was determined by the sound and oral and/or abdominal movements of the infant using video. When the source of the vocalizations could not be attributed to one infant, because multiple individuals were calling or their movements were unclear, it was recorded as "calls by unspecified infant(s)" and excluded from the analysis. The avoidant behaviors of the infants were observed continuously.

Infant retrieval assay with artificially reared infants

Artificially reared infants and their control subjects (artificially reared: n = 4, control: n = 5, PND 2–30, 69 sessions) were repeatedly employed for the retrieval assays in the home cages of their families (Table S8). It was performed twice per week. The detailed procedure is described above. The latency of entering the infant cage, touching the infant, and retrieving the infant was measured. The behaviors of caregivers and infants were analyzed in 0.2 sec bins as shown above. The caregiver's body part where the infant was holding was determined when the infant stopped just after a carrying bout started.

Vocal recordings in isolation

Ten infants (artificially reared male: n = 1 (Michael), control: n = 9 (four males (Cubby, an infant from Chuck's family, and two infants from Oji's family. The three males were not used in the other experiments of the present report), and five females (Eugenie, Shirayuri, Suisen, Mimosa, Kodemari), 170 sessions, PND 3–104) were used for the vocal recordings in isolation.

The recordings were performed once or twice per week. It was conducted in another room separated from the colony rooms. The subject was removed from the home cage and transferred to a plastic cage (28 cm × 44 cm × 20.5 cm) placed in the recording room. The experimenter left the room and recorded the infants' vocalizations using a video recorder and the sound recorder (Sennheiser MKH416-P48U3, TASCAM DR-60DMK II) for 600 sec. The vocalizations were manually analyzed on PRAAT.

Statistical analysis

Statistical analyses were conducted using R software (version 3.6.3) [82]. In the retrieval assays with the normative family, statistical analyses were performed by the generalized linear mixed model (GLMM) with some exceptions. The glmer function of the lme4 package (Bates et al., 2015) was used for the GLMM. The infant and the carrier were added as random effects with an intercept to control pseudoreplication. The fixed effects included in the models are mentioned in the figure legends. The best models were selected using the dredge function in the MuMIn package (Barton 2020). For the correlation matrix, we conducted Pearson's product-moment correlation analysis. Correlation coefficients and *p value*s adjusted with Holm's method or Benjamini–Hochberg's method were calculated using the corr.test function in the psych package (Revelle, W, 2020). For the segmented relationships in regression models, we employed the segmented function in the segmented package (Vito, 2008). The difference in filial behavior was analyzed using the Wilcoxon rank sum test. The Wilcoxon signed-rank test with continuity correction was conducted for the comparison of infant calls for 10 sec after and before the shift of the social contexts.

In the family-reunion observations and the retrieval assays using artificially reared marmosets, statistical analyses were performed by GLMM. The family and the infant nested in the family were added as random effects for family-reunion observations, and the caregiver and the infant were added as random effects for retrieval assays to control pseudoreplication. Random effects included intercepts. PND, artificial rearing, and their interaction were added as a fixed effect in the initial model. The best models were extracted using the dredge function in the MuMIn package (Barton 2020). For count data with/without an upper limit, binomial/Poisson distributions were adopted. Latency data were transformed logarithmically and then treated as Gaussian distributions. Other data were treated with normal distributions. For the analyses of the holding site in the retrieval assays, the clmm function in the ordinal package was employed instead of the glmer function in the lme4 package to deal with the ordinal response variable (Christensen 2019).

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Investigation: SYN, AT, KS, AM, TK, EM
Visualization: SYN, AT, KS
Supervision: KMI, HT, GE, HO, KN, AS, KOK
Writing—original draft: SYN, KOK
Writing—review & editing: SYN, AT, KS, AM, TK, KMI, EM, GE, HO, KN, AS, KOK

SYN designed and carried out the experiments and data analyses with help from TK and EM and produced the tables and figures. SYN designed and carried out experiments described in Fig. 5-6, S3-4, and analyzed data described in Fig 1-6 and S2-4. AT analyzed data described in Fig 1-4, and S1-2. KS designed and carried out experiments described in Fig 1-4 and S1-2. AM carried out a part of experiments, especially those described in Fig. 5, 6, and S4. KOK conceived of and organized the study with AS and with support from KMI, GE, HT, HO, and KN, and wrote the manuscript with SYN and AS, and with contributions from all the authors.

Competing interests:

The authors have no conflicts of interest to declare.

Data and materials availability:

All data are available in the main text or the supplementary materials.

Figures and Tables

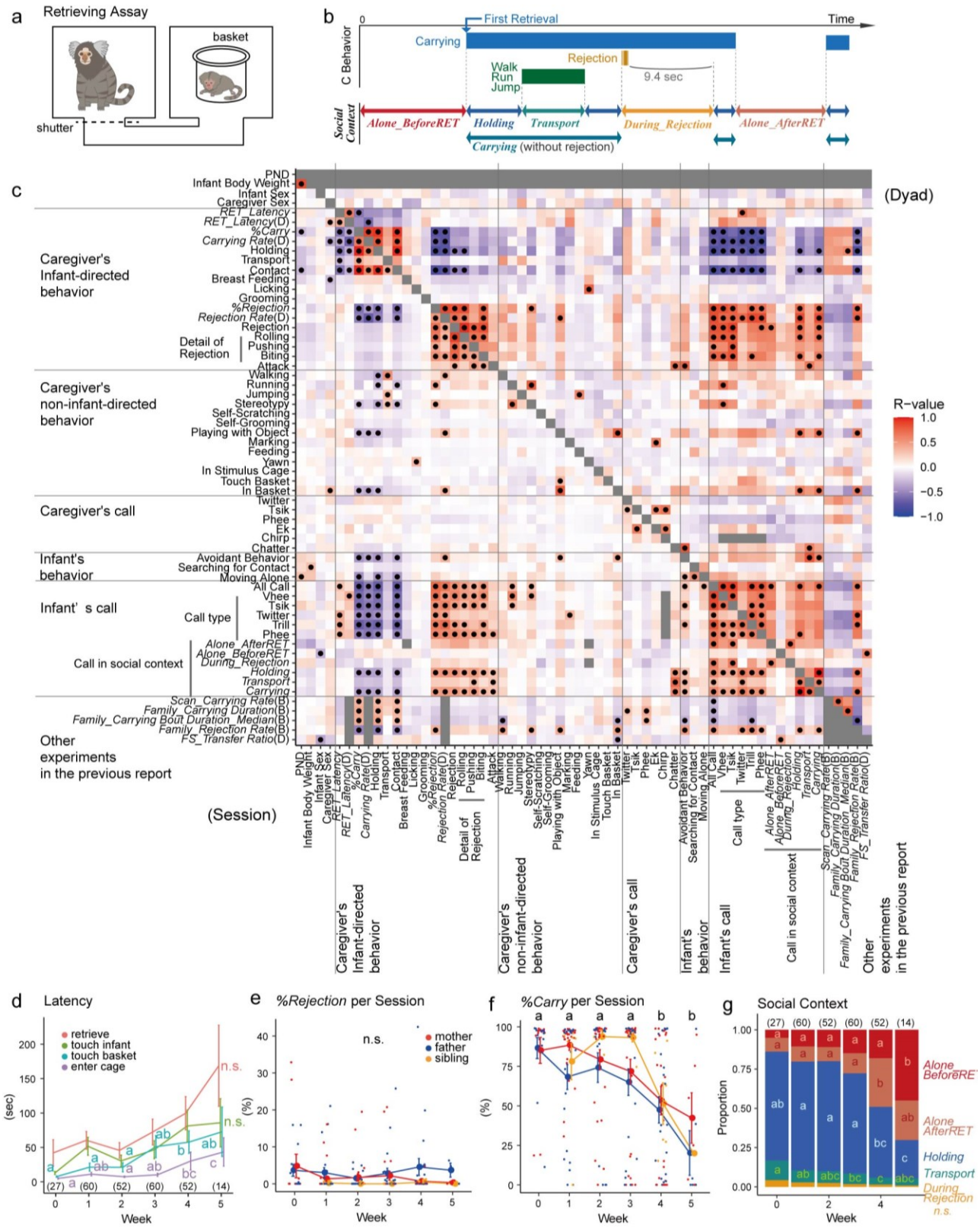


Fig. 1 Caregiver behavior in retrieval assays.

a Schematic of the retrieval assay in the home cage.

b Caregiver-infant interactions and the five social contexts for the infants. Carrying includes transport and holding. A 9.4-second offset in *During_Rejection* was set to include the infant

behaviors directly under the influence of the preceding rejection. The threshold of 9.4 sec was empirically determined in Fig. 2b.

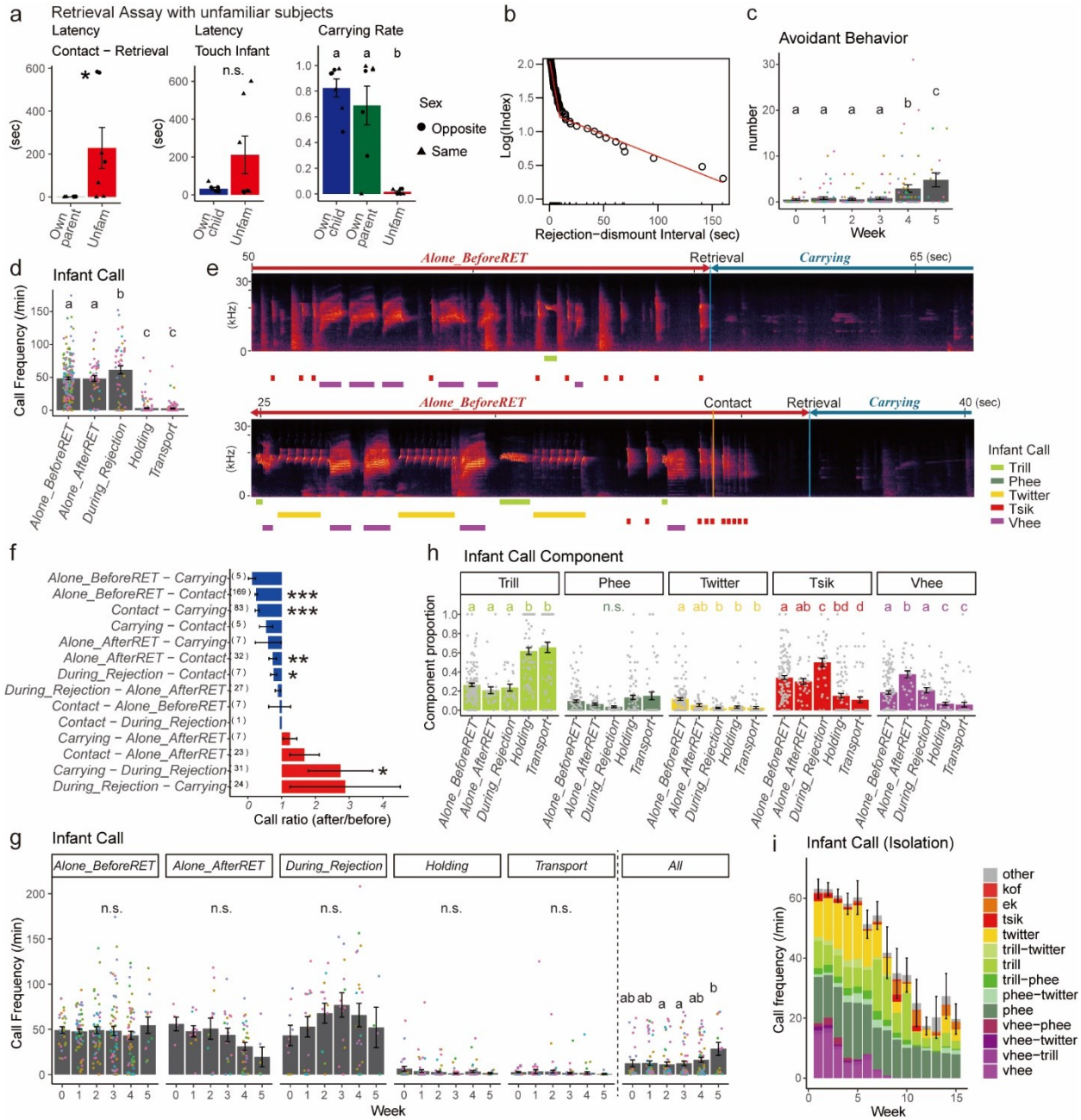
c Correlation matrix of the parameters observed in the retrieval assays. The color indicates correlation coefficients (*r* value, Pearson's product-moment correlation coefficient, see Table S4). Filled circles, $p < 0.05$ (adjusted with Holm's method, see Table S5). The left-bottom and the right-top triangular parts show the parameters calculated per session and the averaged value for each dyad, respectively, and yielded similar results. The bottom five parameters ending with either (B) or (D) are the parenting parameters derived from family observations and food sharing tests in our previous study (Table S3) [45], which are averaged for each dyad (D) or each birth (B).

d Mean ± standard error (s.e.) latencies of the caregiver's behaviors after the opening of the shutter. Red: the first retrieval, yellowish green: the first touch of the infant, blue–green: the first touch of the basket, purple: the first reach of the infant cage. Different letters with the same color indicate significant differences among weeks (GLMM, $p < 0.05$). The numbers within parentheses are the numbers of the sessions.

e-f Mean ± s.e. *%Rejection* (e) and *%Carrying* (f) in each postnatal week (filled circles and error bars). Dots show individual sessions. Red: mothers, blue: fathers, yellow: older siblings. Different letters indicate significant differences among weeks (GLMM, $p < 0.05$).

g Proportion of duration of each social context. Red: Alone before the first retrieval, pink: alone after the first retrieval, blue: holding (carrying the infant without caregiver locomotion), green: transport (carrying the infant with caregiver walking, running, or jumping), yellow: during rejection. Different letters indicate significant differences among weeks (GLMM, $p < 0.05$). The numbers within parentheses are the numbers of the sessions.

Fig. 2 Infant behavior in retrieval assays.



a Retrieval assays using unfamiliar (Unfam) or familiar caregivers (own parent or own infant). Right: The carrying rate was significantly lower in the unfamiliar group than in the familiar groups. The asterisk or different letters indicate $p < 0.05$ in the GLMM.

b Log-survivorship analysis of the rejection-dismount interval. Among 164 observed dismounts, 34 dismounts occurred without a preceding rejection. The other 130 dismounts that occurred after a rejection are plotted for the intervals from the preceding rejection in a serial order. Then, to divide these events into forced and voluntary dismounts, we performed a segmented regression analysis and found the inflection point at 9.4 sec. Red line: the segmented regression line. A total of 83.9% (109 out of 130) of infant dismounting occurred during or within 9.4 seconds after the end of rejection and was regarded as occurring due to the preceding rejection. Voluntary dismounts were rare for infants younger than PND 28 (23.4%, 25 out of 107 cases), while 30 out of 57 cases and 52.6% of dismounts were voluntary on or after PND 28.

c Mean ± s.e. numbers of avoidant behaviors per session. Different letters indicate statistical significance among weeks (GLMM, $p < 0.05$). The dots show the values of each session, and the colors indicate the caregiver.

d Mean ± s.e. frequencies of infant calls in each social context. The dots show the values of each session, and the colors indicate the caregiver. Different letters indicate statistical significance among the social contexts (GLMM, $p < 0.05$).

e Two typical spectrograms of infant calls at the transition from *Alone_BreforeRET* to *Carrying*. After the first contact and retrieval, the calls immediately stopped except for faint trill calls in the bottom panel.

f Mean ± s.e. changes of the call frequency 10 sec before/after the shift of the social contexts (i.e., ratio = 1 means no change). Wilcoxon signed rank test with continuity correction, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. The numbers within parentheses are the numbers of the scenes analyzed.

g Mean ± s.e. call frequencies in each social context. Different letters indicate statistical significance between postnatal weeks (GLMM, $p < 0.05$).

i. Mean ± s.e. total call frequencies and the composition of infant call types in the isolation recording (n = 9, four males and five females).

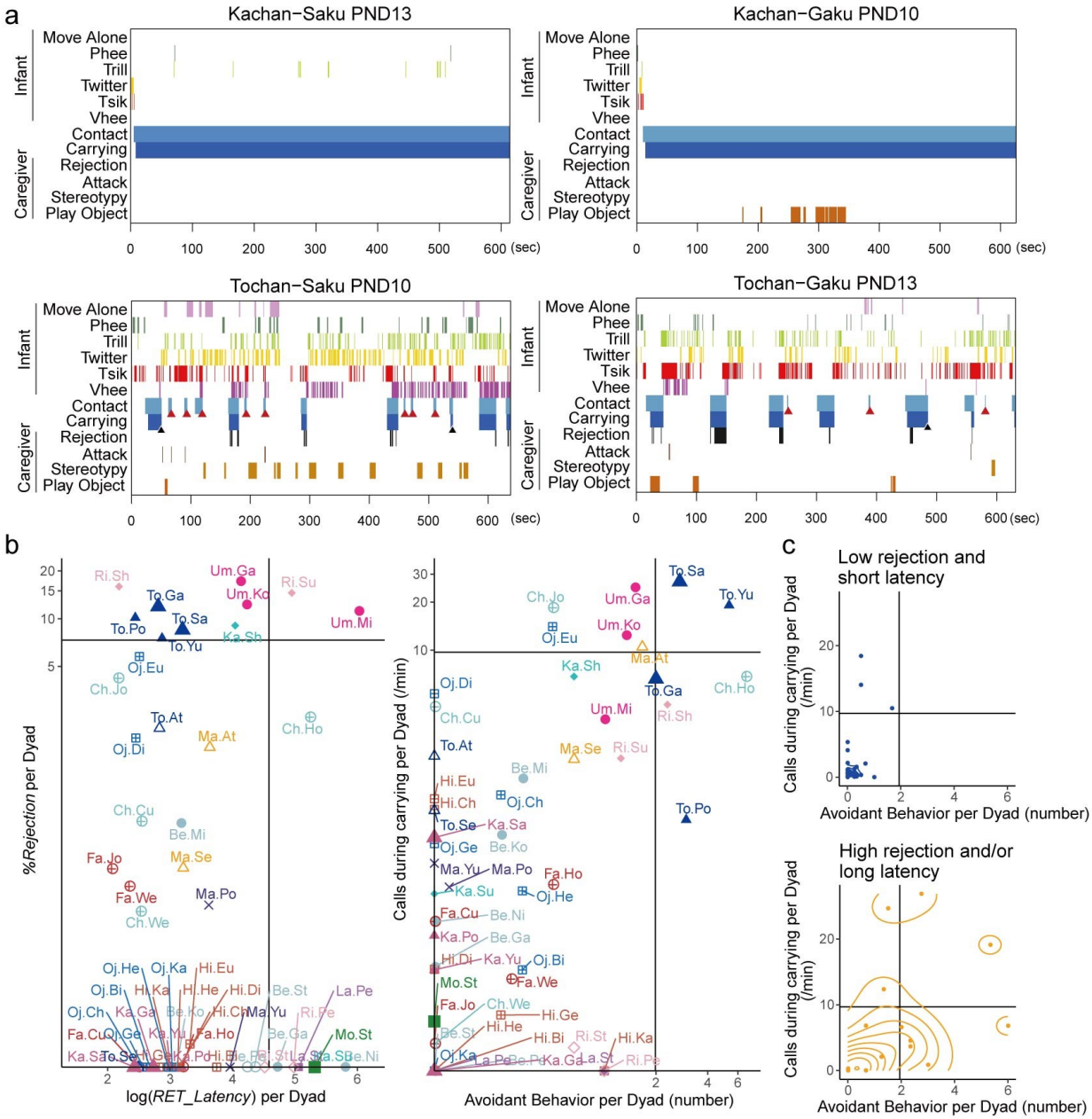


Fig. 3 Relationship between caregiving parameters and infant behaviors.

a Representative raster plots of the retrieval assays using a pair of littermates, Saku and Gaku (PND 10 and 13), by the mother Kachan and the father Tochan. Black triangles: dismounting without preceding rejection within 9.4 sec. Red triangles: refusal to cling when contacted.

b Variation in caregiving (left) and attachment behavior (right) during postnatal weeks 0-3 (54 dyads, excluding one dyad in which the caregiver never retrieved the infant). Each point represents the value averaged for each caregiver-infant dyad, with the color of each caregiver (warm colors: females, cold colors: males). The marker shapes indicate each breeding pair. Labels near markers are the first two letters of the caregiver's and infant's names. The vertical and horizontal lines show the mean + 1 s.d.

c Frequency of infant calls during carrying and the number of avoidant behaviors averaged for each dyad. Upper: both *%Rejection* and the log of *RET_Latency* were less than the mean + 1

s.d. (n = 36), lower: the other dyads (n = 18). Two-dimensional probability density was overlaid as a contour with 10 bins. Vertical and horizontal lines indicate the mean + 1 s.d. for each axis.

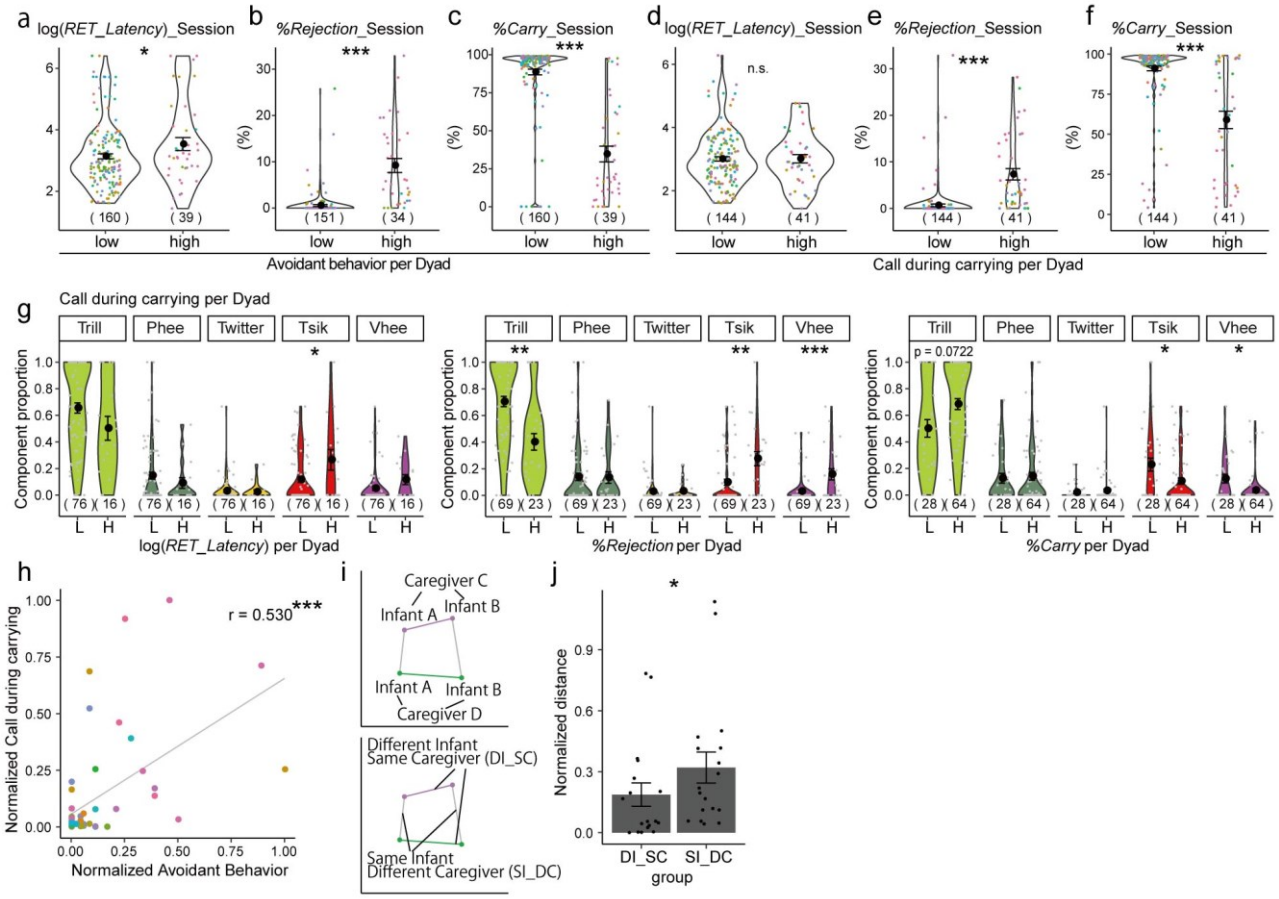


Fig. 4 Infants' tuning of their attachment according to the parenting style of the caregiver.

a-c Violin plots of the parenting parameters (a: *RET_Latency*, b: *%Rejection*, c: *%Carry*) in the groups of dyads divided by high/low infant avoidance, divided by the average value. The numbers within parentheses are the numbers of the sessions during postnatal weeks 0-3; in b, 14 sessions without first retrieval were excluded. The black circles and error bars show the mean ± s.e. Each dot shows the value of each session, with the color indicating each caregiver. GLMM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

d-f Violin plots of the parenting parameters (d: *RET_Latency*, e: *%Rejection*, f: *%Carry*) in the groups of dyads divided by high/low calls during carrying, divided by the average value. The numbers within parentheses are the numbers of the sessions during postnatal weeks 0-3; 14 sessions without first retrieval were excluded. Other indications are the same as in a-c.

g Violin plots of the proportion of infant call types during carrying in each session in the groups of dyads divided by high (H)/low (L) *RET_Latency*, *%Rejection*, and *%Carry* at the average value. GLMM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

h Correlation of infant calls during carrying and avoidant behaviors. These data are the same as those presented in in Fig. 3b; however, both axes have been normalized to 0 to 1 by min-max normalization ((X – min(X)/(max(X) – min(X)) to enable calculation of the distances between points, as shown in Fig. 4i and j. The same color indicates data from the same caregiver. The gray line exhibits a regression line. The calls during carrying and avoidant

behaviors showed a moderate positive correlation ($r = 0.5301$, $p < 0.001$, Pearson's product-moment correlation coefficient).

i Schema showing the method to compare the two attachment parameters of the same caregiver with two different infants (DI_SC, colored segments) and that of the same infant with two different caregivers (SI_DC, gray segments) in the two-dimensional normalized plots of the average of the infant calls during carrying and the avoidant behaviors for each dyad. Each pair of caregivers comprised two individuals with the same relationship to the infant (parents/older siblings).

j Mean ± s.e. lengths of DI_SC (n = 18) and SI_DC (n = 18). Wilcoxon rank sum test, $p = 0.0480$. Each dot represents a distance.

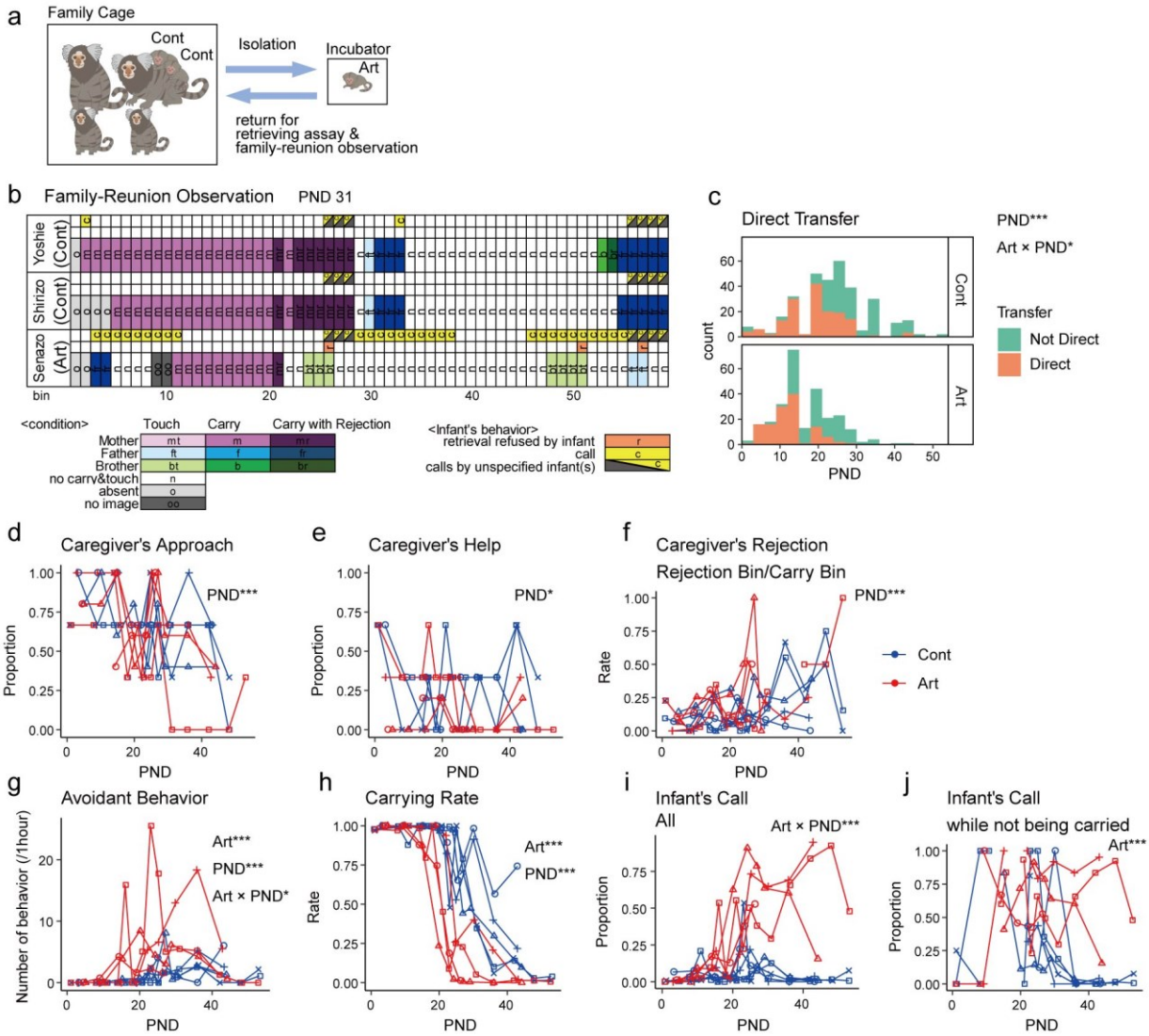


Fig. 5 Family reunion observations with artificially reared infants.

a Schematic of the behavioral tests using artificially reared (Art) and family-reared (Cont) marmosets.

b Representative raster plot of the family reunion observation. Caregivers' and infants' behaviors were coded in a 10-sec bin. “Retrieval refused by the infant” means that an infant did not cling to a caregiver that was in contact.

c Histograms of direct and nondirect transfers from one caregiver to another among the artificially reared infants (Art, $N = 4$, including 265 transfers) and the family-reared infants (Cont, $N = 5$, including 324 transfers). The proportion of direct transfers was lower among older infants ($z = -7.50$, $p < 0.001$). The direct transfers declined earlier among artificially reared infants ($z = -2.27$, $p = 0.0232$).

d-j Summary of the caregivers’ (d-f) and infants’ (g-j) behaviors. The shape of the markers represents each infant. Blue: Cont, red: Art.

d Proportion of the caregivers that approached the cage entrance when the infant was returned to their home cage (96 sessions), which decreased with infant age ($z = -4.51$, $p < 0.001$).

e The proportion of the caregivers that attempted to retrieve the returning infant by the forelimb or showed an aggressive attitude toward the experimenter's hand (96 sessions). The proportion decreased according to the growth of the infants (z = -2.43, p = 0.0149).

f Caregiver's rejection rate as the number of rejecting bins divided by the number of carrying bins (96 sessions). The rejection rate decreased with the growth of the infants (t = 4.992, p < 0.001).

g Numbers of infants' avoidant behaviors, including retrieval refused by the infant and voluntary dismounting (96 sessions). The number of avoidant behaviors was significantly larger among the artificially reared infants (z = 4.80, p < 0.001) and among older infants (z = 4.49, p < 0.001). The increment of avoidant behaviors according to growth was clearer among the control infants (z = -2.39, p = 0.0169).

h Carrying rate calculated by dividing the number of carried bins by the entire bins (96 sessions). The carrying rate was significantly lower among the artificially reared infants (Art) (t = -5.20, p < 0.001) and among the older infants (t = -15.29, p < 0.001).

i Number of bins where infants made any calls in the total attended bins (96 sessions). The proportion increased significantly according to growth in the artificially reared infants (t = 5.30, p < 0.001).

j Number of bins in which the infant made any calls while the infant was not carried (70 sessions). The proportion was significantly higher among the artificially reared infants (t = 4.45, p < 0.001).

Statistical analyses were performed by the generalized linear mixed model (GLMM). *: p < 0.05, **: p < 0.01, ***: p < 0.001.

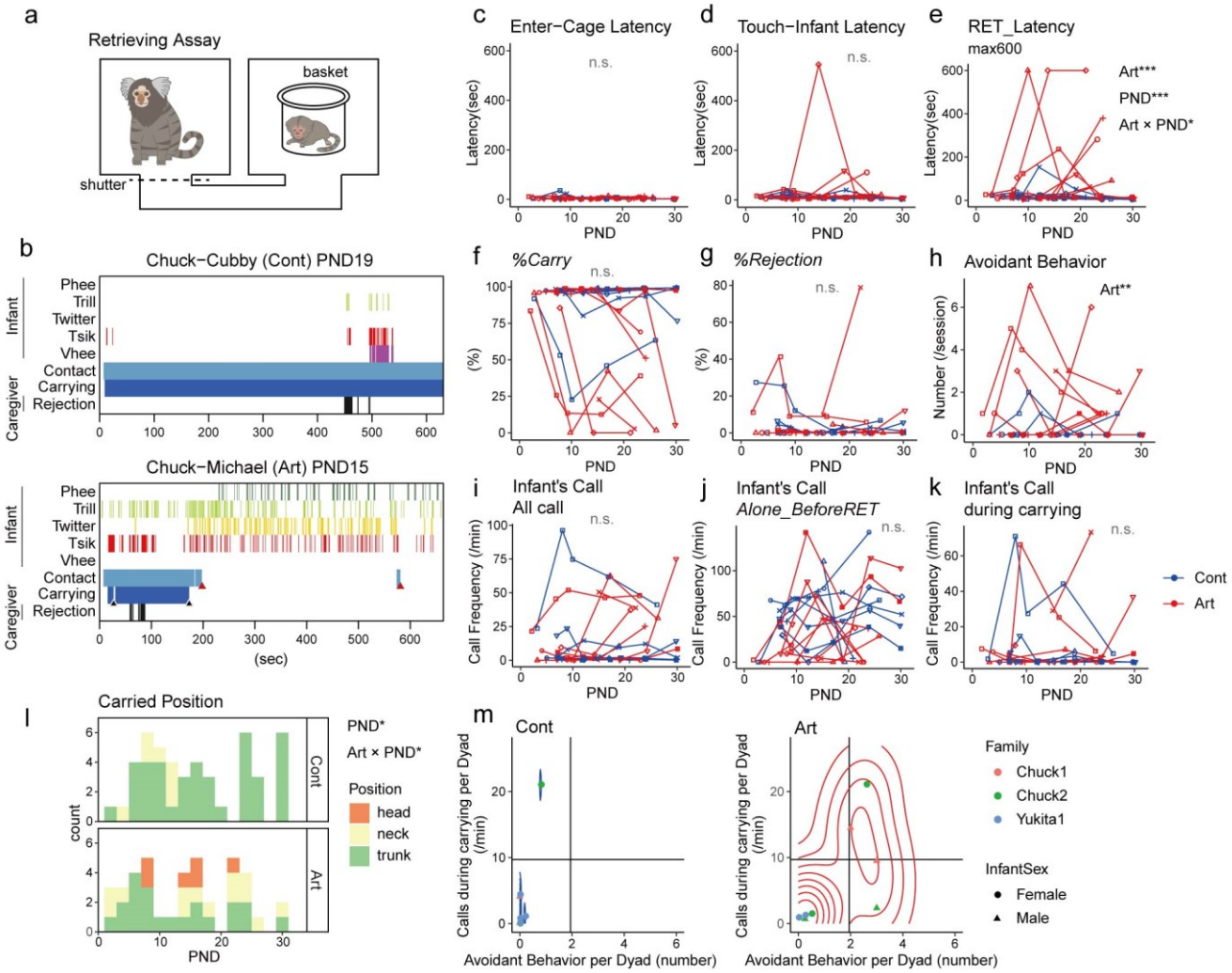


Fig. 6 Retrieval assays with artificially reared infants.

a Schematic of the retrieval assay. The assay was conducted in the home cages. Two cages were connected by a tunnel with a shutter. An infant in a basket was put in a cage, and a caregiver (its mother or father) was placed in the other cage. After the shutter was opened, the caregiver tried to retrieve the infant. The latency at which the caregiver reached the cage where the infant was left (c), touched the infant (d), and retrieved the infant (e) was recorded (69 sessions, Control (Cont): $N = 4$, Artificially reared (Art): $N = 4$).

b Two typical raster plots of the retrieval assays. Cubby (Cont) and Michael (Cubby's littermate, Art) were employed as subjects. Chuck, their father, was utilized as a caregiver in these sessions. While Cubby quickly clung after contact with Chuck and was carried all the time after retrieval, Michael dismounted voluntarily from Chuck without rejection (voluntary dismounting, black arrowhead) and did not cling to Chuck even after contact (retrieval refused by the infant, red arrowhead).

c-k Summary of infants' and caregivers' behaviors. The shape of the markers represents each pair of an infant and a caregiver.

c Latency at which a caregiver reached the cage where an infant was left (69 sessions).

d Latency at which a caregiver touched the infant (69 sessions).

e Latency of retrieving an infant (69 sessions). The latency was longer among the artificially reared infants (t = 3.61, p < 0.001) and the older infants (PND, t = 3.58, p < 0.001). The coefficient of PND was smaller among the artificially reared infants (t = -2.54, p = 0.0111).

f Percentage of carrying (69 sessions).

g Caregiver rejection rate (66 sessions). Three sessions without retrieval within 600 sec were excluded.

h Number of infants' avoidant behaviors (69 sessions). Avoidant behaviors were more frequent among the artificially reared infants (z = 3.64, p < 0.001).

i Frequency of infant calls in the total bins (69 sessions).

j Frequency of the infant's call before the first retrieval (69 sessions).

k Frequency of infant calls while being carried by a caregiver (66 sessions).

L Part of the caregiver's body where an infant clung just after the retrieval (Cont: N = 4, 47 points, Art: N = 4, 40 points). As they grew, the family-reared infants tended to hold on to the caudal part (i.e., trunk) of caregivers (z = 2.01, p = 0.0449). Among the artificially reared infants, this tendency was significantly weak (Art × PND: z = -2.12, p = 0.0342), and they sometimes tried to cling to the head of a caregiver.

m Infant calls during carrying and the number of avoidant behaviors in each dyad. The data points are separately shown for the family-reared infants and the artificially reared infants. Two-dimensional probability density was overlaid as a contour with 10 bins. Vertical and horizontal lines are the same as in Fig. 3b. The same-colored dots indicate the same litter. The shape of the dots shows the infant's sex.

Statistical analyses were performed by the generalized linear mixed model (GLMM). *: p < 0.05, **: p < 0.01, ***: p < 0.001

Supplementary Materials

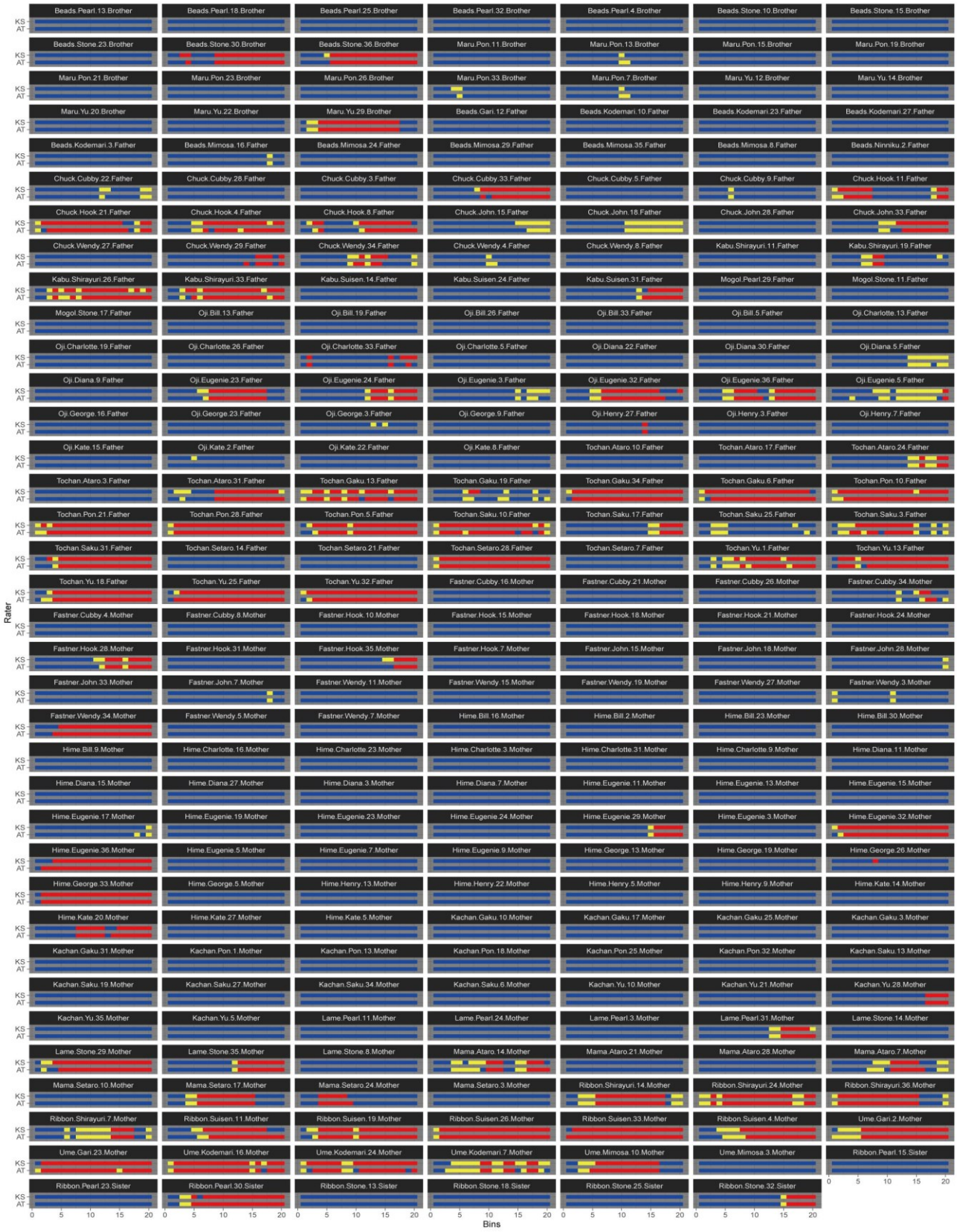


Figure S1 Coincidence between the on-site coarse behavioral coding and off-site, detailed behavioral coding of the infant retrieval assay

The on-site analyses performed with 30-sec bins (by rater S.K. 45) are compared with the off-site analyses with 0.2-sec bins in this study (by rater A.T.), by converting A.T. ‘s coding results into the 30-sec bins and classifying them as “carried” (blue), “rejected” (yellow), or “not carried” (red). The interrater reliability between the two raters was assessed by Cohen’s kappa ($\kappa = 0.926$, $p < 0.001$).

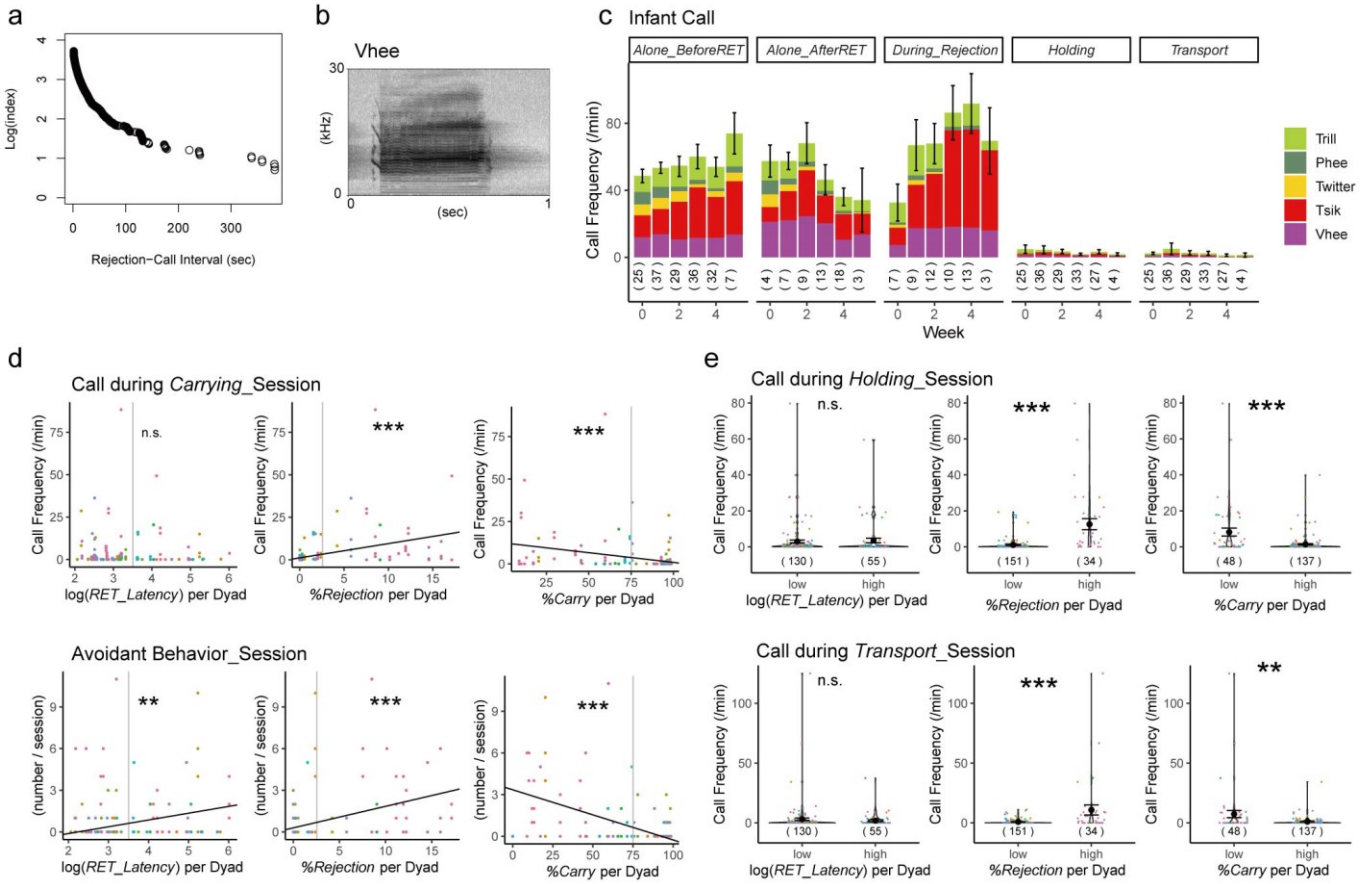


Figure S2 Infant behaviors in the retrieval assay using family-reared infants.

a Segmented regression analysis of the rejection-call interval. The inflection point could not be determined; thus, we also used the 9.4-second offset obtained from movement analysis (Fig. 2b) for call analysis. A total of 77.18% (3981 out of 5158) of the infant calls during carrying occurred during and within 9.4 sec after the preceding rejection.

b Spectrogram of the infant's vhee call.

c Developmental changes in infant call frequencies in each social context. The columns and bars indicate the mean ± s.e. of total calls. The color shows the type of call. This graph corresponds with Fig. 2g. Only the data with detailed vocal analysis were included. The numbers within the parentheses are the numbers of the sessions.

d Scatter plot and regression line of infant calls during carrying (top) and avoidant behavior (bottom) and caregiving parameters (left: Ret_latency, center: %Rejection, right: %Carrying). More avoidant behaviors were observed in the dyad with longer retrieval latency ($t = 2.76$, $p = 0.0078$), although the infant calls during carrying were not different from those in the other dyad. In the dyad with a higher %Rejection, the infant calls during carrying and the avoidant behaviors were more frequent (call-during-carrying: $t = 5.13$, $p < 0.001$, avoidant behavior: $t = 4.323$, $p < 0.001$). In the dyad with a higher %Carrying than the average, the infant calls during carrying and the avoidant behaviors were less frequent (call-during-carrying: $t = -4.25$, $p < 0.001$, avoidant behavior: $t = -7.367$, $p < 0.001$). Each dot shows the value of each session, and the same color indicates data from the same caregiver.

e Violin plots of the infant calls during holding (top) and transport (bottom) in the groups of dyads divided by high/low parental behaviors (retrieval latency, %Rejection, %Carrying). Each dot shows the value of each session, and the color indicates the individual caregiver. The filled circles and error bars show the mean ± s.e. The numbers within parentheses are the numbers of the sessions.

GLMMs were used for statistical analyses. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

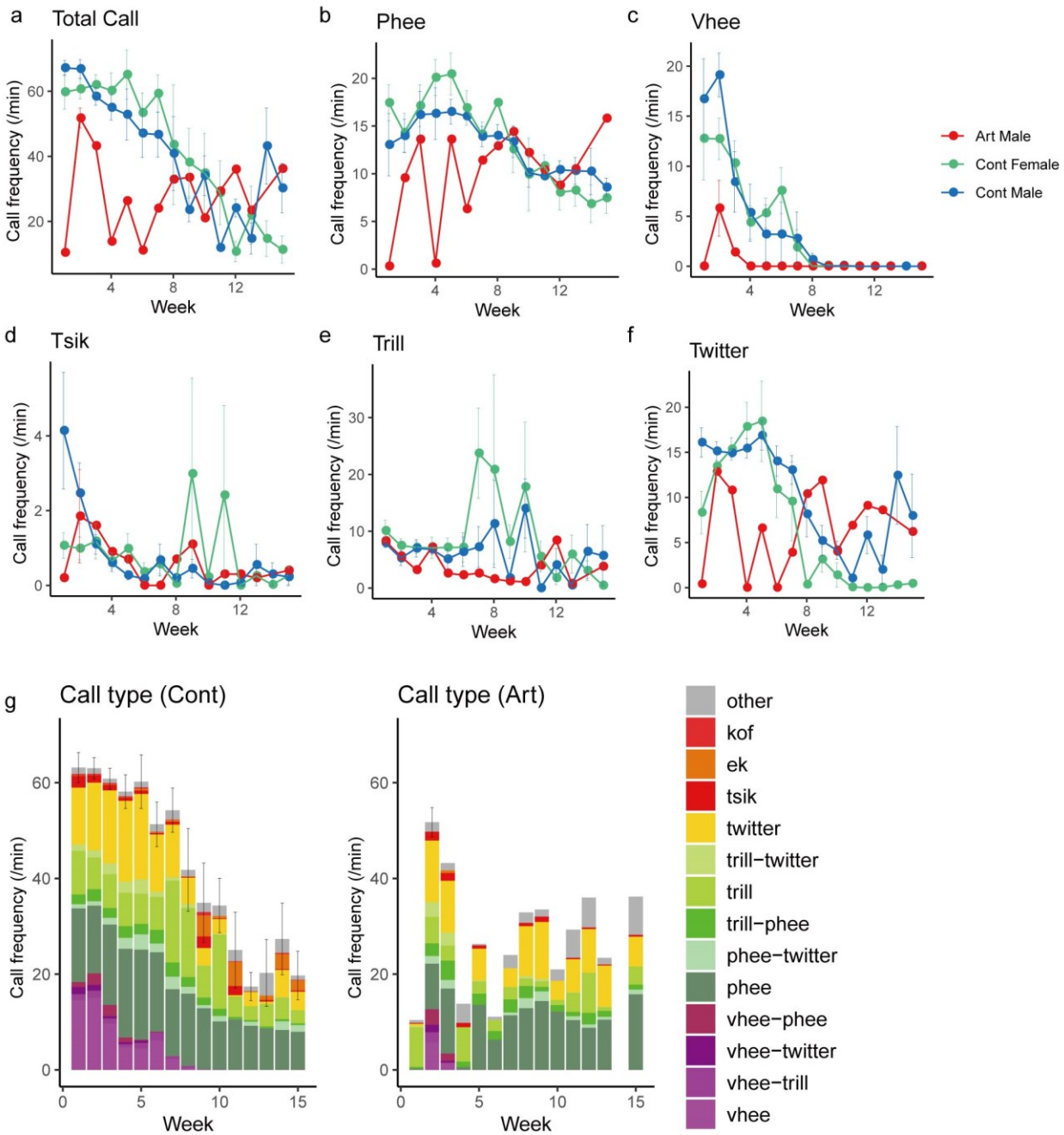


Figure S3 Vocal behaviors in isolation.

a-f The mean ± s.e. call frequencies in the isolated recordings (Art: n = 1 male; Cont: n = 9 (4 males, 5 females). Total calls (a) as well as phee (b), vhee (c), tsik (d), trill (e), and twitter calls (f).

g The mean ± s.e. total calls and composition of call types of Cont (left) and Art infants (right). Error bars represent the standard error of the total calls. The left is the same as in Fig. 2i.

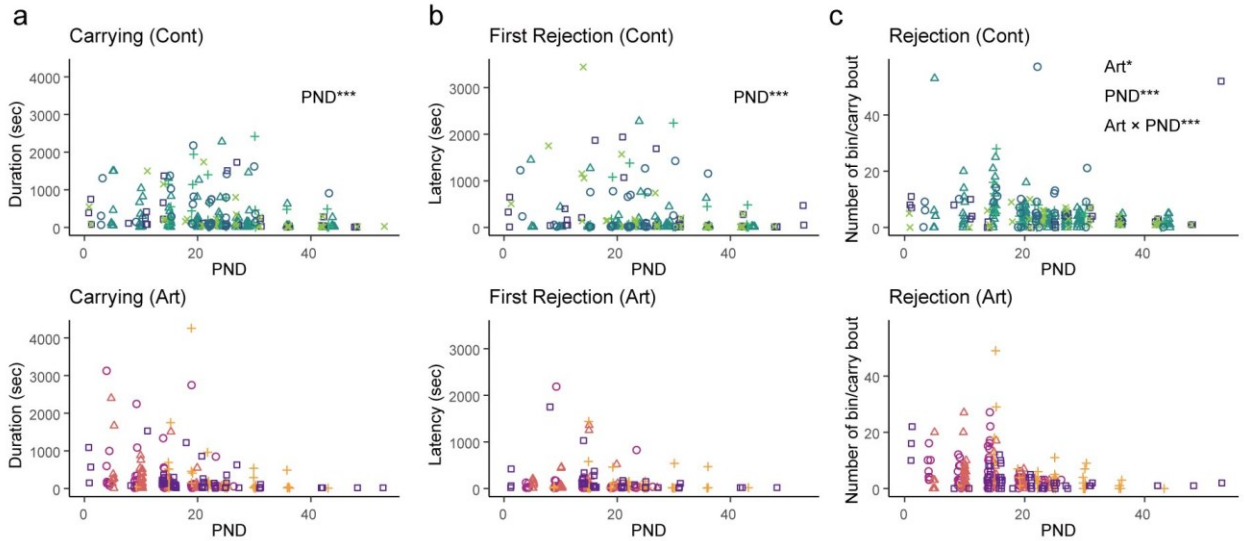


Figure S4 Family reunion observation with artificially reared infants.

The duration of a carrying bout (a), the latency of the first rejection (b), and the number of caregivers' rejection bins (c) in a carrying bout were calculated. A carrying bout is a period from the bin when an infant starts to cling onto a caregiver to the bin when the infant dismounts from the caregiver or moves to another caregiver. The shape and color of the markers represent each infant. Top: family-reared infants (Cont), bottom: artificially reared infants (Art).

a The duration of a carrying bout (Cont: 363 bouts, Art: 299 bouts) was shorter among the older infants ($t = -5.63$, $p < 0.001$).

b The latency of the first rejection in a carrying bout (Cont: 323 bouts, Art: 244 bouts) was shorter among the older infants ($t = -5.10$, $p < 0.001$).

c In the number of caregiver rejection bins (Cont: 363 bouts, Art: 299 bouts), the interaction effect of Art and PND was significant ($z = -7.65$, $p < 0.001$), indicating that the coefficient of PND among the artificially reared infants was smaller than that among the family-reared infants.

Statistical analyses were performed by the generalized linear mixed model (GLMM). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Table S1 List of family-reared infants and their retrieval assay sessions

The numerals indicate the postnatal day of the infant in the session. Parentheses indicate the first week sessions performed by the caregiver without previous experience of infant retrieval assays and were excluded from the statistical analysis. Italics: indicate the sessions without high-quality vocal recordings, which were excluded from the detailed vocal analyses. Underlines indicate the initial 16 sessions that were censored at 300 sec when the first retrieval did not take place, while the others were censored at 600 sec. Red: female, blue: male. See also [45].

Infant	Birth No.	Mother		Father		Sibling1		Sibling2	
Name	in parents	Name	Test (PND)	Name	Test (PND)	Name	Test (PND)	Name	Test (PND)
Hook	1	Fastener	*(3), 7, 10, 15, 18, 21, 24, 28, 31, 35*	Chuck	*(4), 8, 11, 16, 19, 21, 24, 28, 32, 35*				
Wendy	2	Fastener	3, 5, 7, 11, 15, 19, 27, 34	Chuck	4, 8, 27, 29, 34				
John	3	Fastener	7, 15, 19, 28, 33	Chuck	15, 19, 28, 33				
Cubby	4	Fastener	4, 8, 16, 21, 26, 34	Chuck	3, 5, 9, 22, 28, 33				
Bill	1	Hime	*(2), 9, 16, 23, 30*	Oji	*(5), 13, 19, 26, 33*				
Kate	1	Hime	*(5), 14, 20, 27*	Oji	*(2), 8, 15, 22*				
George	2	Hime	5, 13, 19, 26, 33	Oji	3, 9, 16, 23				
Charlotte	2	Hime	3, 9, 16, 23, 31	Oji	5, 13, 19, 26, 33				
Diana	3	Hime	3, 7, 11, 15, 27	Oji	5, 9, 22, 30				
Henry	3	Hime	5, 9, 13, 22	Oji	3, 7, 27				
Eugenie	4	Hime	3, 5, 7, 9, 11, 13, 15, 17, 19, 23, 24, 29, 32, 36	Oji	3, 5, 23, 24, 32, 36				
Pon	1	Kachan	*(1), 13, 18, 25, 32*	Tochan	*(5), 10, 21, 28, 35*	Maru	*(3), (5), 7, 11, 13, 15, 19, 21, 23, 26, 33*		
Yu	1	Kachan	*(5), 10, 21, 28, 35*	Tochan	*(1), 13, 18, 25, 32*	Maru	*(4), (6), 12, 14, 20, 22, 29*		
Gaku	2	Kachan	3, 10, 17, 25, 31	Tochan	6, 13, 19, 34				
Saku	2	Kachan	6, 13, 19, 27, 34	Tochan	3, 10, 17, 25, 31				
Ataro	1	Mama	7, 14, 21, 28	Tochan	3, 10, 17, 24, 31				
Setaro	1	Mama	(3), 10, 17, 24, 31	Tochan	7, 14, 21, 28, 35				
Stone	1	Lame	*8, 14, 21, 29, 35*	Mogol	*(3), 11, 17, 24, 31*	Beads	*10, 15, 23, 30, 36*	Ribbon	*(4), 13, 18, 25, 32*
Pearl	1	Lame	*(3), 11, 17, 24, 31*	Mogol	*8, 14, 21, 29, 35*	Beads	*(4), 13, 18, 25, 32*	Ribbon	*10, 15, 23, 30*
Mimosa	1	Ume	(3), 10, 23, 27, 31	Beads	8, 16, 24, 29, 35				
Kodemari	1	Ume	7, 16, 24, 29, 35	Beads	3, 10, 23, 27				
Ninniku	2			Beads	2, 23				
Gari	2	Ume	2, 23	Beads	12				
Shirayuri	1	Ribbon	7, 14, 24, 31, 36	Kabu	(4), 11, 19, 26, 33				
Suisen	1	Ribbon	4, 11, 19, 26, 33	Kabu	7, 14, 24, 31, 36				

Table S2 List of observed behaviors in the retrieval assay.

	Item	Description
Caregiver's behavior	Carrying	Infant clung to caregiver's body
	Holding	Carrying without caregiver's locomotion
	Transport	Carrying with caregiver's locomotion
	Contact	Physical contact of body parts between infant and caregiver
	Breast feeding	Breast feeding (only by mother)
	Licking	Licking infant
	Grooming	Manipulating infant's fur by hand or teeth
	Rejection	Rolling + Pushing + Biting
	Rolling	Rubbing infant on cage wall/floor during carrying
	Pushing	Pushing or scratching infant during carrying
	Biting	Biting infant during carrying
	Attack	Biting or scratching infant without carrying
	Walking	Walking
	Running	Running
	Jumping	Jumping
	Stereotypy	Repeated vertical circling more than three times
	Self-scratching	Scratching own body parts by hand or limb
	Self-grooming	Grooming own body parts
	Playing with object	Manipulating objects by hand or biting objects
	Marking	Rubbing anogenital area on cage or objects
	Feeding	Masticating food pellets
	Yawning	Yawning
	In stimulus cage	Staying in stimulus cage with basket
	Touching basket	Touching basket
	In basket	Staying in basket
Infant's behavior	Avoidant behavior	Voluntarily dismounting without rejection or more than 9.4 sec after the last rejection & not carried even with body contact with caregiver (continuous recording)
	Searching for contact	Approaching caregiver while alone
	Infant moving alone	Moving alone without contact or carrying
Call	Twitter	Rapid series of elements, regularly and closely spaced and each rising swiftly in frequency. Territorial call that can also indicate alertness or agitation within the group. Neutral call.
	Tsik	Short call that rises slightly before dropping straight down to a much lower pitch. Made when marmoset is alarmed. Negative call.
	Phee	Long call with constant pitch. Within-group contact call. Neutural call.
	Trill	Call with cyclically fluctuating frequency. Within-group contact call. Neutral~positive call.
	Ek	(Caregiver only) Low-pitched and short call that is uttered singly or several in close succession. Indicates mild anxiety. Negative call.
	Chirp	(Caregiver only) Short and quiet call that falls from high to low frequency. Made in friendly/affiliative contexts and when near a favored food. Positive call.
	Chatter	(Caregiver only) Low-pitched, harsh and staccato call. Aggressive call. Negative call. Infant's chatter-like calls were classified as repeated tsiks.
	Vhee	(Infant only) Long and broadband call used to get attention from other group members. Negative call.
Social context	*Alone_BeforeRET*	Infant is not carried before the first retrieval
	Alone_AfterRET	Infant is not carried after the first retrieval (except "*During_Rejection*")
	During_Rejection	Period from the start of rejection to 9.4 seconds after the end of rejection
	Holding	Infant is carried without caregiver's locomotion (except "*During_Rejection*")
	Transport	Infant is carried with caregiver's locomotion (except "*During_Rejection*")
	Carrying	*Holding* + *Transport*

Table S3 List of parameters employed in our previous paper.

These parameters were obtained in [45] and were used for the correlation matrix in Fig. 1c.

Assay	Item	Description
Infant retrieval assay	*RET_Latency*	The latency of the first retrieval
(On site, 30-sec bins)	*Carrying Rate*	The rate at which a caregiver carried an infant
	Rejection Rate	The rate of bins with rejection in carrying bins
Instantaneous scan sampling of the family cage	*Scan_Carrying Rate*	The rate at which a caregiver carried any infant
(Littermates were not distinguished)		
Continuous family observation	*Family_Carrying Duration*	Total duration of carrying
(Littermates were not distinguished)	*Family_Carrying Bout Duration_Median*	The median duration of a carrying bout
	Family_Rejection Rate	The rate of bins with rejection in carrying bins
	FS_Transfer Ratio	The ratio at which an infant was given a food item by a caregiver when the infant approached the caregiver with the food item

Table S4 The r values of the correlation matrix in Fig. 1c.
Red and blue indicate positive and negative correlations, respectively.
(This Table is provided in an excel file.)

Table S5 The p values of the correlation matrix in Fig. 1c.
Red: $p < 0.05$.
(This Table is provided in the excel file file.)

Table S6 List of artificially reared infants.

Name	Sex	Family (Father) Name	Family Cage	Retrieval Assay	Vocal Recording in Isolation	Reason of Artificial Rearing	Housing condition	Comment
Michael	M	Chuck		✓	✓	Born as a twin, but one nipple of the mother did not work. We have tried to expose and reunite with the original family, but did not succeed, at least partly because both parents were inherently rejective toward any infants. We stopped indirect family exposure through a mesh wall after Michael's finger was bitten by the family member at the mesh.	Singly housed in an incubator in the same room with their family and other marmosets, with occasional exposure to the family. Contacted with a family caregiver during infant retrieval assays (10-15 min, 0-3 times /week).	
GeorgeD	M	Chuck	✓	✓		Born as a twin but one nipple of the mother did not work.	Singly housed in an incubator in the same breeding room with its family. Reintroduced repeatedly to the family (2-6hr/day, 3-7times/week)	
Atako	F	Chuck	✓	✓		Triplet	Singly housed in an incubator in the same breeding room with their family and step family (Chuck). Reintroduction to Chuck's family (2-6hr/day, 3-7times/week)	Chuck's family was assigned for the family exposure for Atako, because the biological family was engaged in the other experiment.
Senazo	M	Yukita	✓			triplet	Singly housed in an incubator in a separate room from its family. Reintroduced repeatedly to the family (3-6hr/day, 3-7times/week)	
Senako	F	Yukita	✓	✓		triplet	Singly housed in an incubator in a separate room from its family. Reintroduced repeatedly to the family (3-6hr/day, 3-7times/week)	

Table S7 List of family reunion observations with Art infants.

Name	Belonging	Reared Condition	Sex	DOB	Family (Father) Name	Num. of Elder Siblings	Date of Experiments		PND		Num. of Experiments	Cage Size
							From	To	From	To		
Shirizo	RIKEN	Control	M	2017/11/17	Yukita	1	2017/11/18	2018/1/9	1	53	15	42 × 65 × 60 cm
Yoshie	RIKEN	Control	F	2017/11/17	Yukita	1	2017/11/18	2018/1/9	1	53	15	42 × 65 × 60 cm
Senazo	RIKEN	Artificial Reared	M	2017/11/17	Yukita	1	2017/11/18	2018/1/9	1	53	15	42 × 65 × 60 cm
Nana	RIKEN	Control	F	2019/6/15	Chuck	3	2019/6/17	2019/7/29	2	44	9	43 × 66 × 60 cm
GeorgeD	RIKEN	Artificial Reared	M	2019/6/15	Chuck	3	2019/6/17	2019/7/29	2	44	9	43 × 66 × 60 cm
Atako	RIKEN	Artificial Reared	F	2019/6/16	Chuck	3	2019/6/17	2019/7/12	1	26	6	43 × 66 × 60 cm
Shirie	RIKEN	Control	F	2019/10/10	Yukita	1	2019/10/11	2019/11/22	1	43	9	42 × 65 × 60 cm
Yoshimi	RIKEN	Control	F	2019/10/10	Yukita	1	2019/10/11	2019/11/22	1	43	9	42 × 65 × 60 cm
Senako	RIKEN	Artificial Reared	F	2019/10/10	Yukita	1	2019/10/11	2019/11/22	1	43	9	42 × 65 × 60 cm

Table S8 List of retrieval assays with Art infants.

"Cage size" represents the size of a home cage. In the test, two home cages were connected by the tunnel.

					Father		Mother		Date of Experiments		PND		
Name	Belonging	Reared Condition	Sex	DOB	Name	Num. of Experiments	Name	Num. of Experiments	From	To	From	To	Cage Size
Cubby	RIKEN	Control	M	2016/3/31	Chuck	2	Fastener	3	2016/4/14	2016/4/21	14	21	43 × 66 × 60 cm each
Michael	RIKEN	Artificial Reared	M	2016/3/31	Chuck	2	Fastener	3	2016/4/8	2016/4/22	8	22	43 × 66 × 60 cm each
Nana	RIKEN	Control	F	2019/6/15	Chuck	5	Fastener	4	2019/6/18	2019/7/11	3	26	43 × 66 × 60 cm each
GeorgeD	RIKEN	Artificial Reared	M	2019/6/15	Chuck	4	Fastener	4	2019/6/18	2019/7/11	3	26	43 × 66 × 60 cm each
Atako	RIKEN	Artificial Reared	F	2019/6/16	Chuck	5	Fastener	4	2019/6/18	2019/7/9	2	23	43 × 66 × 60 cm each
Shirie	RIKEN	Control	F	2019/10/10	Yukita	6	Yuki	5	2019/10/13	2019/11/9	3	30	42 × 65 × 60 cm each
Yoshimi	RIKEN	Control	F	2019/10/10	Yukita	6	Yuki	5	2019/10/13	2019/11/9	3	30	42 × 65 × 60 cm each
Senako	RIKEN	Artificial Reared	F	2019/10/10	Yukita	6	Yuki	5	2019/10/13	2019/11/9	3	30	42 × 65 × 60 cm each

Supplementary movie 1 Typical retrieval in the retrieval assay
Saku (PND 13) was left alone and called severely, and then Kachan (mother) retrieved Saku. After the retrieval, Saku stopped calling.

Supplementary movie 2 Caregiver's rejection in the retrieval assay
Gaku (PND 13) was carried and then rejected by Tochan (father). After the rejection, Gaku dismounted and called intensely while alone.

Supplementary movie 3 Direct transfer of family reunion observation
Yoshie (PND 1) was rejected by Junior (elder brother) and called severely. Yukita (father) approached them (0:18) and then received Yoshie from Junior (0:23). After Yukita's retrieval, Yoshie stopped calling.

Supplementary movie 4 Dismounting of infants during carrying in family reunion observation
The Art infant Senazo (PND 23) clung to the father (Yukita) (0:14) and soon dismounted without distinct rejection (0:23). Then, the Cont littermate Yoshie clung to Yukita (0:32) and was rejected (0:40~). Yoshie kept clinging during the rejection for more than 50 sec and finally dismounted (not included in this video).

Supplementary movie 5 First retrieval of a control infant in the retrieval assay
After the first retrieval, the Cont infant (Cubby, the littermate of Michael, PND 15) quickly moved to the back of the father (Chuck).

Supplementary movie 6 First retrieval of an artificially reared infant in the retrieval assay
After the first retrieval, the Art infant Michael (PND 15) moved onto the body of the father (Chuck) (0:42~). Michael occasionally clung to Chuck's head and face, which induced rejection by Chuck (0:55~). Michael tooka longer time to settle to a suitable position than Cubby.

Supplementary movie 7 Avoidant behavior of an artificially reared infant in the retrieval assay.
The Art infant Michael (PND 15) clung to the trunk of the father Chuck and dismounted without distinct rejection (0:19). Chuck tried to retrieve Michael again (0:31), but Michael refused to cling. This movie is from the session at the bottom of Fig. 6b (approximately 150-190 sec).