

Brain size, life history, and metabolism at the marsupial/placental dichotomy

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The evolution of mammalian brain size is directly linked with the evolution of the brain's unique structure and performance. Both maternal life history investment traits and basal metabolic rate (BMR) correlate with relative brain size, but current hypotheses regarding the details of these relationships are based largely on placental mammals. Using encephalization quotients, partial correlation analyses, and bivariate regressions relating brain size to maternal investment times and BMR, we provide a direct quantitative comparison of brain size evolution in marsupials and placentals, whose reproduction and metabolism differ extensively. Our results show that the misconception that marsupials are systematically smaller-brained than placentals is driven by the inclusion of one large-brained placental clade, Primates. Marsupial and placental brain size partial correlations differ in that marsupials lack a partial correlation of BMR with brain size. This contradicts hypotheses stating that the maintenance of relatively larger brains requires higher BMRs. We suggest that a positive BMR–brain size correlation is a placental trait related to the intimate physiological contact between mother and offspring during gestation. Marsupials instead achieve brain sizes comparable to placentals through extended lactation. Comparison with avian brain evolution suggests that placental brain size should be constrained due to placentals' relative precociality, as has been hypothesized for precocial bird hatchlings. We propose that placentals circumvent this constraint because of their focus on gestation, as opposed to the marsupial emphasis on lactation. Marsupials represent a less constrained condition, demonstrating that hypotheses regarding placental brain size evolution cannot be generalized to all mammals.

encephalization | maternal energy hypothesis | altricial | basal metabolic rate

Mammalian brain size is widely relevant to neuroscience. Aside from the classical interpretation of brain size as a proxy for cognitive or sensorimotor capacity (1–3), brain size corresponds to the macroscopic, microstructural, and connective composition of the mammalian brain with remarkable accuracy (4, 5). However, despite decades of research, the factors underlying the evolution of brain size relative to body size (referred to as “brain size” hereafter), particularly relative brain enlargement, remain a matter of debate (6). Maternal life history investment traits, such as gestation and litter size, are generally accepted as mammalian brain size correlates (7–13) and are considered to reflect a female's ability to energetically provision her offspring's brain growth (14, 15–17). A more controversial potential brain size correlate is basal metabolic rate (BMR) (18, 19). Recent studies have confirmed a consistent correlation between increased brain size and BMR across placentals (20, 21), although this does not apply to all placental clades (8, 22).

Interpretations as to why BMR should influence mammalian brain size vary. It has been suggested that high BMR is required for brain maintenance, because brain tissue is metabolically active and costly to run, so that either BMR needs to increase (“metabolic constraints hypothesis”) (23–25) or other metabolically active tissues (e.g., the gut) need to decrease (“expensive tissue hypothesis”) (26, 27) to allow for the evolution of a larger brain.

Martin (16) was the first to suggest that maternal BMR may act synergistically with maternal investment parameters to supply energy for offspring brain growth. His “maternal energy hypothesis” posits that, aside from brain size increases mediated by extended maternal investment, such as gestation or lactation, the transfer of metabolic energy during maternal care increases with increased BMR, thereby allowing for growth of a larger brain in a shorter time. Isler and van Schaik (15) recently elaborated on this suggestion, showing that maternal investment–related brain size correlates differ between altricial (immature-born) and precocial (mature-born) placentals, whereas BMR is correlated with brain size in both groups. Based on these findings, these authors developed the “expensive brain hypothesis,” which allows for different life history correlates of brain size, with BMR playing a dual role of allowing both the growth and the maintenance of a larger brain.

The well-established relevance of maternal investment parameters for brain size, particularly with respect to neonatal maturity, points toward brain ontogeny as a key component of brain size evolution. Indeed, the extent of prenatal and postnatal brain growth and structural maturity differ greatly between altricial and precocial placentals (28–33). Neonatal brain size does not correlate with adult brain size, however (28, 30). This is puzzling, because birds, in which prehatching and posthatching brain growth patterns also vary according to neonatal maturity, tend to be relatively larger-brained with increasing altriciality (28, 30, 34). Thus, it is unfortunate that the interactions between maternal investment and brain size have been largely researched on the most precocial mammalian radiation, placentals (28, 35, 36).

Marsupials and placentals have independently increased brain size since their divergence at least 125 million years ago (37), and this provides an important avenue for testing paradigms of mammalian brain size evolution that have thus far been based largely on placentals. Marsupials display a specialized mode of reproduction, differing from placentals in those life history parameters considered most relevant for the evolution of mammalian brain size; all species give birth to highly altricial neonates that are minute compared with maternal body mass (38). Marsupial maternal investment focuses on lactation, which lasts up to 20 times longer than gestation, whereas gestation rarely exceeds 1 mo and can be as short as 12 d (39). In contrast, placental gestation times range from 2 wk to 2 y, and the lactation period is usually less than one-half as long as (and rarely exceeds three times as long as) the gestation period (40).

The neonatal marsupial brain is less mature than the placental fetal brain of similar size (41). After birth, marsupial brain development (including most of neurogenesis, i.e., neuron precursor formation; refs. 41–43) continues to proceed slowly during the ex-

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tended postnatal life in the pouch (41, 44). These specialized ontogenetic characteristics have been suggested to constrain marsupial brain size (42, 45). The impact of the radically different marsupial life history and brain ontogeny has not been quantified, however, because relatively little data on marsupial brain size (mostly on Australian marsupials and didelphids; refs. 46 and 47) have been collected. This lack of interest might be related to the persistent notion that marsupials are small-brained (1, 45, 48), despite evidence to the contrary (46, 49, 50).

In an important recent contribution, Ashwell (49) published the largest and most diverse dataset of marsupial brain size to date, containing 198 species. In this paper, we use Ashwell's dataset, as well as data from previous studies of placental mammals (17, 51), to provide a direct quantitative comparison of marsupial and placental brain size evolution, using identical bivariate and multivariate analyses for both clades. We revisit the contention that marsupials are systematically smaller-brained than placentals, and assess scaling differences and interactions among brain size, BMR, and maternal life history investment traits in marsupials and placentals. We discuss our results in the context of the striking differences in life history and brain ontogeny patterns between the two clades and compare these with the dichotomy of altricial and precocial birds.

Results

Encephalization Quotient Comparisons. Comparisons of encephalization quotients (EQs) between major therian clades revealed a broad overlap between marsupials and placentals. The brain sizes of most marsupial clades largely resembled those of placentals (Fig. 1). This was confirmed by Wilcoxon rank-sum tests (Table S1), showing that differences between marsupials and placentals are concentrated in comparisons between the relatively smallest-brained marsupials, Diprotodontia and Peramelemorpha, and the largest-brained placentals, Laurasiatheria and especially Euarchontoglires. However, the high mean EQ of Euarchontoglires is due mostly to large-brained primates, and removing Primates from this dataset largely removes this effect (Fig. 1 and Table S1). Without Primates, Euarchontoglires is much smaller-brained (Fig. 1) and is significantly larger-brained only than Peramelemorpha. It should be noted that there are no significant differences between Didelphimorphia and any placental clade or between Dasyuromorphia and any pla-

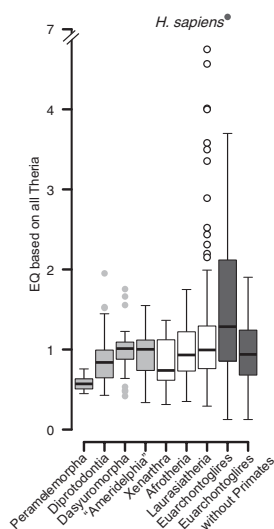


Fig. 1. Boxplots of EQ values in therian superorders. Error bars represent SD. Marsupials are not systematically smaller-brained than placentals, but Euarchontoglires is significantly larger-brained than most other therian superorders, due largely to the inclusion of Primates in this clade. The y axis is condensed between 4 and 7 to show the large EQ of *Homo sapiens*.

cental clade other than Euarchontoglires (and this difference disappears when Primates are excluded; Table S1).

Regressions Against Body Size. Log-log regressions of brain size against body weight revealed significant scaling differences between marsupials and placentals (Fig. 2A). The intersection point between regression lines suggests that marsupials weighing <43 g, although falling within the distribution of placentals, are on average larger-brained than similar-sized placentals. This was confirmed by an exact Wilcoxon rank-sum test of brain size values divided by body size values, showing that placentals <43 g ($n = 172$) have smaller brains for their body size on average than marsupials ($n = 41$; Wilcoxon rank sum test statistic $W = 2,422.5$; $P = 0.004$). Monotreme brain size is above the marsupial regression line, and Echidnas have comparatively large brains even with respect to placentals.

A comparison of BMR versus body size in marsupials and placentals based on the dataset use here, obtained from McNab (52), showed that marsupial BMR overlaps with that of placentals but overall is lower and less variable (Fig. 2B). Small marsupials have particularly low BMRs. Monotreme BMRs are far below the re-

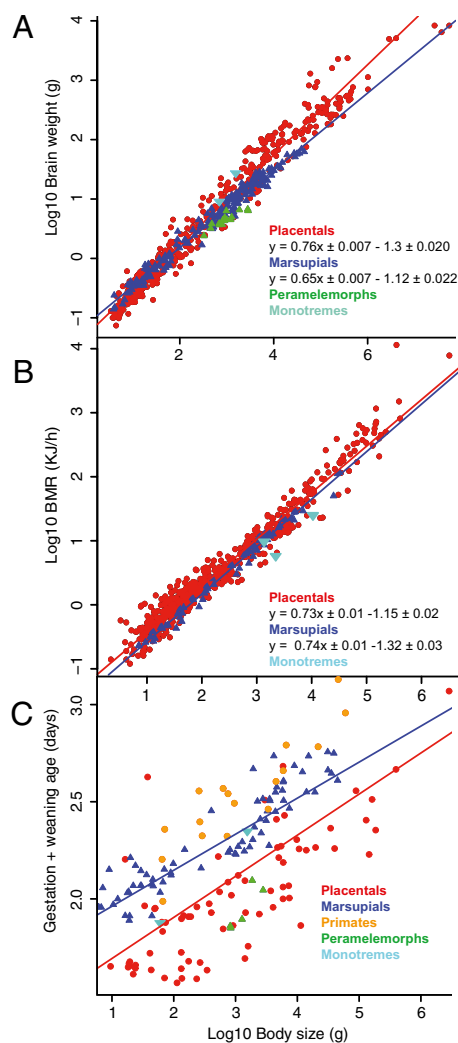


Fig. 2. Regressions of (A) log brain size (in g; marsupials, $n = 198$; placentals, $n = 493$) with the marsupial Peramelemorpha shown separately; (B) log BMR (in KJ/h; marsupials, $n = 68$; placentals, $n = 546$); and (C) log maternal investment time (gestation + weaning in days; marsupials, $n = 76$; placentals, $n = 91$) against log body size (in g), with Peramelemorpha and the placental clade of Primates shown separately.

gression line for marsupials and are within the range of those mammals displaying the lowest BMR. The pygmy shrew *Sorex araneus* has by far the highest residual on the BMR–body size regression. Because *S. araneus* is also one of the smallest species in the placental dataset, it represents a high-leverage outlier, and thus was not included for further analysis in the partial correlation dataset.

Regressions of total maternal investment times (gestation + weaning age) revealed that marsupials are distinguished from similar-sized placentals by consistently longer-lasting maternal investment times, as demonstrated by their significantly higher intercept in the regression of overall maternal investment time versus body size (Fig. 2C). Similar results were obtained when only weaning age was regressed (data not shown). Primates were the only placental clade whose overall investment time resembled that of marsupials. Monotreme maternal investment fell between that of marsupials and placentals, with platypuses more similar to placentals and echidnas resembling marsupials.

Partial Correlations. The partial correlation analyses (Fig. 3 and Table S2) revealed that marsupial brain size is correlated with weaning age and litter size only. Marsupial BMR was not correlated with any other trait except for a marginally significant negative correlation with weaning age, which disappeared after phylogenetic correction; similarly, a positive correlation between gestation and weaning age disappeared after phylogenetic correction. Placental brain size also was significantly correlated with weaning age and litter size and weakly but significantly correlated with BMR. The correlation between BMR and brain size was the least changed in magnitude by phylogenetic correction. Furthermore, placentals differed from marsupials in exhibiting a negative correlation of gestation length and litter size and a strong positive correlation between gestation and weaning age that was robust to phylogenetic correction. Placental BMR also was significantly negatively correlated with gestation time in the phylogenetically uncorrected data. Partial correlations using residuals of life history traits based on body mass data published with BMR values, rather than those published with brain size values, did not significantly affect results (Table S3).

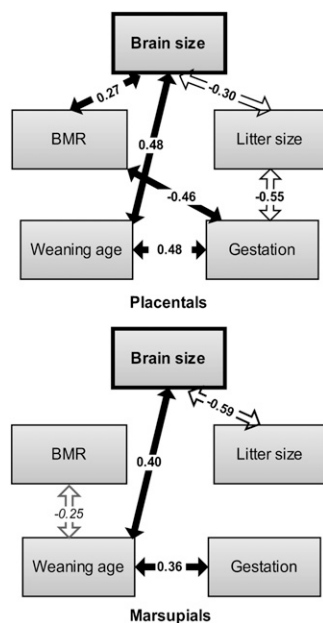


Fig. 3. Flow diagrams of significant partial correlations of body size-adjusted gestation, weaning age, litter size, BMR, and brain weight. Empty arrows indicate negative correlations; numbers in italics indicate marginal significance ($P = 0.05–0.1$).

Analysis of the extended marsupial dataset excluding gestation did not yield different results (Table S4).

Residual Regressions with Brain Size. Bivariate regression analyses of body size–adjusted residuals of all of the variables (Table 1) largely confirmed the partial correlation analysis results. Litter size and weaning age explained most of the variation in residual brain size of both clades in uncorrected and phylogenetically corrected analyses. The regression between placental BMR and brain size was only marginally significant in uncorrected regression due to some strong outliers (e.g., primates, particularly *Homo sapiens*), but was significant at $P = 0.02$ in the phylogenetically corrected analyses. Aside from this, phylogenetic correction dramatically reduced R^2 values, particularly in placentals. Regressions using residuals of life history traits based on body masses published with BMR values did not affect the results (Table S5).

Discussion

Relative Brain Size in Marsupials and Placentals. Regressions of marsupial and placental brain size against body size confirmed that the brain size of marsupials is not systematically smaller than that of similar-sized placentals (49, 50, 53). Moreover, small marsupials not only overlap with placentals in relative brain size (see also ref. 49), but also are larger-brained on average than similar-sized placental species (which might explain the outstanding performance of tiny dasyurids in cognition tests; ref. 54). A comparison of EQs confirmed that major marsupial and placental clades broadly overlap (49), particularly when Primates is excluded from Euarchontoglires. This suggests that much of the perceived brain size discrepancies between placentals and marsupials stems from inclusion of the exceptionally large-brained Primates and perhaps also the mostly large-bodied and small-brained Diprotodontia. Because Diprotodontia are the most speciose and charismatic of marsupials, their smaller EQs have perhaps contributed disproportionately to the misconception of a systematic brain size difference between marsupials and placentals. (For further analysis of the evolution of brain size scaling within marsupial clades, see ref. 49.) Interestingly, the early-diverging placental clades Xenarthra (including armadillos, sloths, and anteaters) and Afrotheria (including elephants, hyraxes, manatees, aardvarks, and tenrecs) have relatively low EQs, further confirming that large brain size is not a general feature of Placentalia. Rather, our results support paleontological studies suggesting that the exceptionally large brains observed only in some placental lineages have evolved independently from a smaller-brained ancestor (55, 56).

Table 1. Bivariate regression, uncorrected and based on phylogenetic independent contrasts (PICs) of body size-adjusted brain size residuals against body size-adjusted residuals of all four parameters included in the partial regression to assess the amount of variation in brain size explained by each variable

	Uncorrected				PIC			
	Slope	SE	R ²	P	Slope	SE	R ²	P
Marsupials								
BMR	-0.010	0.215	-0.023	0.962	-0.234	0.227	0.026	0.309
Gestation	0.170	0.100	0.062	0.100	0.109	0.115	0.001	0.827
Weaning age	0.390	0.164	0.306	0.000***	0.373	0.098	0.206	0.001**
Litter size	-0.325	0.056	0.435	0.000***	-0.381	0.091	0.243	0.001**
Placentals								
BMR	0.323	0.020	0.039	0.104	0.374	0.008	0.107	0.008**
Gestation	0.401	0.099	0.217	0.000***	0.238	0.107	0.072	0.030*
Weaning age	0.548	0.072	0.476	0.000***	0.223	0.076	0.121	0.005**
Litter size	-0.484	0.082	0.365	0.000***	-0.286	0.069	0.213	0.000***

Asterisks indicate the level of significance: 0.1 > P > 0.05; *0.05 > P > 0.01; **0.01 > P > 0.001; ***P < 0.001.

Interactions of Brain Size with Maternal Life History Investment Traits. The partial correlations among life history traits and BMR in marsupials and placentals largely agree with previous analyses of these variables (39, 40, 57). Our findings also confirm previous suggestions that marsupial BMR–body size relationships exhibit very low variance and that residuals are only weakly, if at all, correlated with reproductive traits (58, 59).

Our residual regression results suggest that litter size explains a large amount of the variation in residual brain size both before and after phylogenetic correction in marsupials (see also ref. 15) and placentals, so that this variable emerges as the strongest brain size correlate in therian mammals. This relationship has been widely documented in placentals (8, 15, 51) and reflects the metabolic cost of offspring brain growth to the mother as part of the trade-off between offspring number and offspring “quality” (39, 57). The impact of lactation on brain size is also well known in placentals, in which extended postnatal parental investment of any kind (including, e.g., alloparental care) has been shown to favor larger brains (15, 35). A good example of this is the very large-brained Primates, the only placental clade with maternal investment durations consistently similar to those of marsupials.

Although placentals exhibited no significant partial correlation of gestation time and brain size, the two parameters were significantly correlated in the residual regressions. It is possible that the partial correlation was obliterated, because gestation is correlated with brain size only in precocial placentals (15), which were not treated separately in our analysis. Thus, marsupials specifically resemble altricial placentals in their lack of a brain size–gestation correlation. In marsupials, the lack of a brain size–gestation correlation might also be explained by the fact that marsupial gestation appears to be confined to less than one estrus cycle (60), which may make it a relatively unattractive selection target for larger brain size.

BMR and Brain Size. A relatively weak but stable correlation of body size-adjusted BMR and brain size values was found in placentals, consistent with previous studies (15, 16) and with similar R^2 values as reported in a study on a larger placental sample (20). In contrast, none of our analyses showed evidence of a positive correlation between brain size and BMR in marsupials. In fact, the smallest marsupials, with very low BMRs, are on average larger-brained than similar-sized placentals. The lack of a correlation between metabolic turnover and marsupial brain size (see also ref. 49) argues against suggestions that high BMR is required for maintenance of a large mammalian brain (15, 24). It may be argued that marsupials’ large brain size could have evolved at the expense of other metabolically expensive tissue, particularly the gut (15, 26, 27); however, the range of dietary adaptations of the marsupial gut resembles that of placentals (61, 62), and dietary classifications based on placental intestinal proportions apply to marsupials as well (63). Thus, consistently shorter marsupial guts related to energetic demands of the brain seem unlikely.

Further evidence against a universal limiting influence of BMR on mammalian brain size comes from the earliest-diverging extant mammalian radiation, the monotremes. Echidnas (*Tachyglossus aculeatus*) in particular have low BMRs, but have brain sizes similar to those of relatively large-brained placentals (64; see also Fig. 2). However, energy consumption of brain tissue per unit time is clearly higher than that of other tissues, making brains metabolically expensive to run (26, 65). This is consistent with the fact that the only two radiations of vertebrates to evolve large brains—mammals and birds—have exceptionally high BMR compared with other vertebrates. Thus, high BMR seems to be a prerequisite for the evolution of large brain size, but the BMR values of extant mammals appear to exceed that required for minimum brain maintenance. The considerable encephalization of echidnas suggests that this threshold was crossed at the latest in the common ancestor of crown mammals in the early Jurassic, when monotremes diverged from therians (66).

Our results suggest that a linkage of brain size and BMR (15–17) is a typical trait of placentals, or at least of some placental clades (8). The placental reproductive focus on gestation could explain this pattern, because it results in immediate physiological contact between mother and offspring through the placenta. This is thought to allow for increased maternal energy transfer per unit time at higher BMRs in placentals, but not in marsupials (67). Thus, in placentals, prenatal brain size increase can occur with either greater metabolic input per unit time through increased BMR or longer metabolic investment through increased gestation (see also refs. 15, 16, 21, and 67). The negative BMR–gestation correlation supports this notion, suggesting that increased BMR can offset shorter gestational periods and vice versa (see also refs. 17 and 51). In contrast, marsupial placentation occurs for only a few days toward the end of gestation (60), which explains why our partial correlation analyses suggest that the metabolic cost of marsupial brain development is met solely through litter size reduction and extended lactation periods. Moreover, lactating marsupials transfer less metabolic energy per unit time to their offspring compared with lactating placentals (68), suggesting that large brain size can evolve in mammals with small metabolic budgets as long as extended brain growth under maternal care is possible. This relationship is particularly well represented by the relatively small-brained peramelemorphs, who also have very short maternal investment periods. Monotremes, which lack a placenta, are expected to fall into the marsupial pattern.

The polarity of a possible link between long gestation period and a positive correlation of BMR with brain size represents an intriguing issue. Recent studies of genomic imprinting in the mammalian placenta (69) and evolution of regulatory genes (70) have led to the tentative suggestion that extended intrauterine life may be a derived trait of placentals. This suggests that the short gestation of marsupials and monotremes represents a plesiomorphic state (despite the fact that marsupial reproduction is otherwise highly specialized; ref. 60), and that a BMR–brain size correlation may be a derived placental trait.

Developmental Basis of Brain Size Correlates. The vastly different maturity of marsupials and placentals at birth provides insight into the structural connection between brain size correlates and brain ontogeny. Avian brain development represents an interesting point of comparison in this respect because it resembles that of mammals. In both clades, altricial species have smaller and less mature brains after shorter incubation or gestation times (28, 33, 71); postnatal or posthatching brain growth is more extensive (28, 31, 32) and continues longer than growth in utero or in ovo (34). However, unlike mammals, altricial birds are larger-brained as adults compared with precocial birds (28, 34). It has been suggested that posthatching brain growth in precocial birds is constrained by the greater structural maturity of the brain required for independent life immediately after hatching. In contrast, an extended period of posthatching care in altricial birds is thought to allow for less mature hatchling brains, permitting more extensive postnatal growth and overall larger brains (14, 34).

To explain the lack of an avian-like brain size dichotomy in mammals, it has been suggested that postnatal brain growth in altricial mammals might be constrained by a very low incidence of postnatal neurogenesis (18, 28). However, this hypothesis ignores the fact that extensive postnatal neurogenesis occurs in all marsupials. Interestingly, it has been suggested that increased mammalian neonatal brain maturity represents a constraint on postnatal brain growth, as in birds (72). Thus, placentals, as the more precocial mammalian clade, would be expected to be constrained to smaller brain size compared with the more altricial marsupials. However, due to the long placental gestation times, the bulk of placental brain growth occurs in utero before structural maturity is required for neonatal survival, such that postnatal brain growth may become less important for increases in brain size. An outline of this hypothesis is depicted in Fig. 4. This scenario is consistent with the fact that ex-

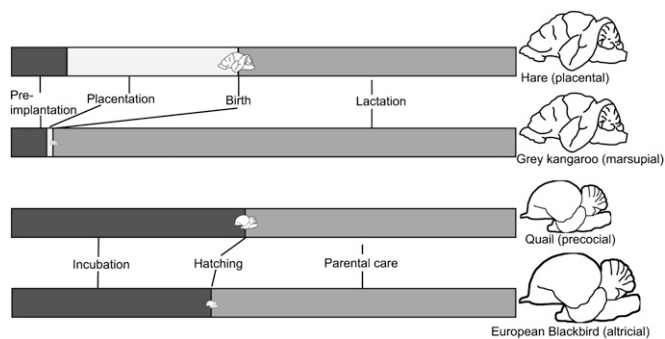


Fig. 4. Schematic presentation of the relationships among maternal investment periods, neonatal maturity, and brain size proposed in this study. In placentals and precocial birds, neonatal/hatching brain size is larger after a longer period of gestation or incubation. Brain growth of marsupials and altricial birds is achieved predominantly through extended lactation or post-hatching care. Placentals have an extended period of placentation, which allows for more extensive prenatal brain growth compared with marsupials and precocial birds. The relative size of adult and neonatal/hatching brain cartoons, and the relative durations, correspond to actual data for the species depicted (except for the neonatal brain of the gray kangaroo, which would have been too small to be visible). Hares and gray kangaroos have similar encephalizations. Data on brain sizes and durations are from various sources (17, 31, 72, 74).

tended gestation is correlated with brain size in precocial placentals, but not in altricial placentals (15). This suggests that extensive intrauterine life indeed allows for the evolution of larger brain size in precocial mammals. In contrast, precocial birds achieve relatively greater neonatal maturity through increased yolk provisioning, but lack the continuous gestational maternal energy provisioning (and the possibility of further augmentation of brain size through increased BMR) available to placentals. Thus, the fact that avian, but not mammalian, brain size is affected by neonatal maturity may be due to the considerable reproductive differences between the two clades.

Conclusion. Our results confirm several hypotheses of mammalian brain size evolution—in particular, the prediction of the maternal energy hypothesis that large-brained mammals with lower BMRs should have extended maternal investment times. In addition, our inclusion of marsupials provides further insight into the patterns of mammalian brain size evolution by showing that placental brain size evolution represents a unique case among mammals, connected with the placental reproductive emphasis on gestation. Based on this, several avenues for further research arise. If BMRs exceed brain maintenance rates in extant mammals, investigation of brain size in mammalian ancestors will provide clues as to when (and perhaps how often) the minimum BMR to allow a mammalian-sized brain evolved. Due to the close interaction between reproduction-related brain size correlates and brain ontogeny, an improved understanding of brain growth and structural development patterns in species with different reproductive strategies emerges as another important area of future research. Our results emphasize that factors influencing the evolution of brain size are complex and emerge from fields that are traditionally researched separately, such as physiology, developmental biology, zoology, and paleontology. The integration of such interdisciplinary research represents the most appropriate avenue for providing a comprehensive evolutionary background for neurobiological research.

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Materials and Methods

A dataset of brain and body weights of 197 marsupials was compiled from Ashwell (49). Data for 457 placentals were provided by Martin (51). EQ values (i.e., the residual of a species on log-log a brain–body size regression of all species; ref. 1) for all species were calculated based on least squares regressions of brain size and body size of all species available, using R (73). EQs of major therian clades (placental superorders and marsupial orders) were compared using pairwise Wilcoxon rank-sum tests, Holm-adjusted for multiple comparisons, for a general assessment of how relative brain sizes of major Therian clades compare. EQ comparisons have been criticized because they ignore scaling differences between mammalian clades (3); however, EQ comparison represents a convenient tool for illustrating clade-specific relative brain sizes (32). In addition, using EQ allows for straightforward nonparametric between-clade comparisons, which is more appropriate given the differing sample sizes within the clades.

Bivariate least squares regressions of logged brain size, BMR (marsupials, $n = 69$; placentals, $n = 543$), and maternal investment length (gestation time + weaning age; marsupials, $n = 77$; placentals, $n = 91$) against body mass were conducted separately for marsupials and placentals to compare the scaling of these traits in the two clades. Gestation, weaning age, and litter size data used for placentals were largely those included in the brain size dataset used by Martin (17, 51), supplemented by data from the Animal Aging & Longevity Database (AnAge) (74). Gestation, weaning age, and litter size data for marsupials were obtained from Fisher et al. (39); if several values were listed, their average was used. Placental BMR values were taken from McNab (52). For comparison, monotreme data (52, 64, 74, 75) also were incorporated into the regression plots.

Partial correlation analyses and bivariate regression analysis were conducted with 45 marsupials (Table S6) and 69 placentals for which data on brain size, parameters reflecting maternal investment (i.e., gestation, weaning age, and litter size; litter size data were obtained from the AnAge database for placentals and from Fisher et al. (40) for marsupials), BMR, and body size were available. Because the extremely short duration of marsupial gestation suggests that gestation might not exert a strong influence on marsupial brain evolution, an additional dataset excluding gestation length was compiled for this clade for partial correlation analysis. This increased the sample size to 52 marsupial species. To avoid a confounding influence of body size, residuals from log-log regressions against body size were used for all variables. Both brain size and BMR are generally recorded with body size as a reference, whereas maternal investment parameters are recorded per species and theoretically should be independent of intraspecific body size variation. Thus, the partial correlation analyses were conducted on residuals of brain size and BMR regressed against the body masses with which they were published. Residuals of gestation, weaning age, and litter size were obtained from regression of both brain-associated and BMR-associated body sizes, and alternative partial correlations were conducted to assess whether this changed the partial correlation significances.

Partial correlation and bivariate regression analyses of data adjusted by body size from the brain size dataset also were conducted after computation of phylogenetic independent contrasts (PICs) of the datasets, to address potential confounding effects of phylogenetic nonindependence (76). The contrasts were created on a composite phylogeny (see Fig. S1 for the phylogeny and S1 Text for sources) with equal branch lengths of 3 Ma (77) using the Mesquite package PDAP (78, 79). Equal branch lengths (80) and a compound phylogeny were preferred over a dated supertree (81), because the topology was not well resolved for the species used in our analysis, and because the tree dating and topology are controversial (82). Regression of absolute values against the SDs of the contrasts was not significant in all cases, confirming that the contrasts were standardized appropriately (83). Two high-leverage contrast outliers within primates (*Homo sapiens*/*Pan troglodytes* and *Alouatta palliata*/*Callithrix jacchus* + *Cebuella pygmaea*, both displaying extensive brain size differences) and one within marsupials (*Burramys parvus*/*Cercartetus concinnus*, with *C. concinnus* exhibiting unusually long gestation periods for marsupials) were removed.

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