Fossil Evidence on Origin of the Mammalian Brain

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Many hypotheses have been postulated regarding the early evolution of the mammalian brain. Here, x-ray tomography of the Early Jurassic mammaliaforms *Morganucodon* and *Hadrocodium* sheds light on this history. We found that relative brain size expanded to mammalian levels, with enlarged olfactory bulbs, neocortex, olfactory (pyriform) cortex, and cerebellum, in two evolutionary pulses. The initial pulse was probably driven by increased resolution in olfaction and improvements in tactile sensitivity (from body hair) and neuromuscular coordination. A second pulse of olfactory enhancement then enlarged the brain to mammalian levels. The origin of crown Mammalia saw a third pulse of olfactory enhancement, with ossified ethmoid turbinals supporting an expansive olfactory epithelium in the nasal cavity, allowing full expression of a huge odorant receptor genome.

Brain size and sensory faculties diversified dramatically as mammals evolved to fill an immense variety of ecological niches, and much attention has been devoted to reconstructing the organization and origin of the ancestral mammalian brain. Among living taxa, mammals have the largest brains relative to body size and are unique in possessing the neocortex (isocortex) (Fig. 1). Accordingly, research has focused on origin of the neocortex (1–5) and evolutionary increases in brain size [measured as a function of body mass, or "encephalization quotient" (EQ) (6, 7)].

Mammalia arose in or before the Early Jurassic [~200 million yeas ago (Ma)] (8–11). The oldest fossils are mostly tiny isolated jaws and teeth, and until now the rare skulls offered little detail on early brain evolution because internal access required destructive sampling. Comparative and developmental anatomy of living mammals has been our chief source of information. Such studies postulated numerous drivers for increased encephalization and origin of the neocortex, including innovations in hearing, feeding, taste, olfaction, miniaturization, parental care, endothermy, elevated metabolism, and nocturnality (1–7). Although deeply informative, few details have emerged on timing or sequences of historical events.

Here, we ask what sequence of evolutionary events culminated in the origin of the mammalian brain, and how was the brain in the ancestral mammal different from its closest extinct relatives? For this study, we used high-resolution x-ray computed tomography (12) to nondestructively scan tiny fossil skulls of two basal mammaliaforms from the Early Jurassic of China (Fig. 1), *Morganucodon oehleri* (9–11) and *Hadrocodium wui* (13). As a test of postulated neurobiological drivers, we digitally extracted casts of their endocranial cavities (endocasts), which closely approximate the size and shape of the brain, and

compared them with endocasts of seven more primitive fossils and 27 crown mammals (14). The scans yielded digital measurements and anatomical details (Fig. 2) that offer a nuanced sequence of historical events in early brain evolution.

The mammalian lineage (Synapsida) diverged from other tetrapods in the Carboniferous (~300 Ma) (15). The braincase initially lacked fully ossified walls and floor; hence, little is known of early brain form, and EQ estimates are imprecise. The first detailed view of the premammalian brain is seen in basal Cynodontia, a clade originating in the Late Permian (~260 Ma)

that includes living mammals and their proximate extinct relatives. The cynodont endocranial cavity is more fully enclosed, with EQs initially measuring from ~0.16 to 0.23 (Fig. 3) (16–20). The olfactory bulbs were small (12), and the nose lacked ossified turbinals. The forebrain was narrow and featureless, the midbrain exposed dorsally, and the pineal eye persisted. The cerebellum was wider than the forebrain, and the spinal cord was narrow (12). The middle ear ossicles remained massive and attached to the lower jaw, and the cochlea occupied only a shallow bony recess (16, 21, 22). Compared with their living descendants, early cynodonts possessed low-resolution olfaction, poor vision, insensitive hearing, coarse tactile sensitivity, and unrefined motor coordination. Sensory-motor integration commanded little cerebral territory.

Morganucodon is the basal-most member of Mammaliaformes, a clade including mammals and their closest extinct relatives (9–11, 13, 15). It records a first major pulse in encephalization with an EQ of ~0.32, which is nearly 50% larger than in basal cynodonts (Fig. 3). The olfactory bulb and olfactory (pyriform) cortex are by far the regions of greatest expansion (Fig. 2). A deep annular fissure encircles the olfactory tract, marking a distinctive external division of the mammalian brain between the olfactory bulb and cortex. The cortex is inflated and wider than the cerebellum, covering the midbrain and the pineal
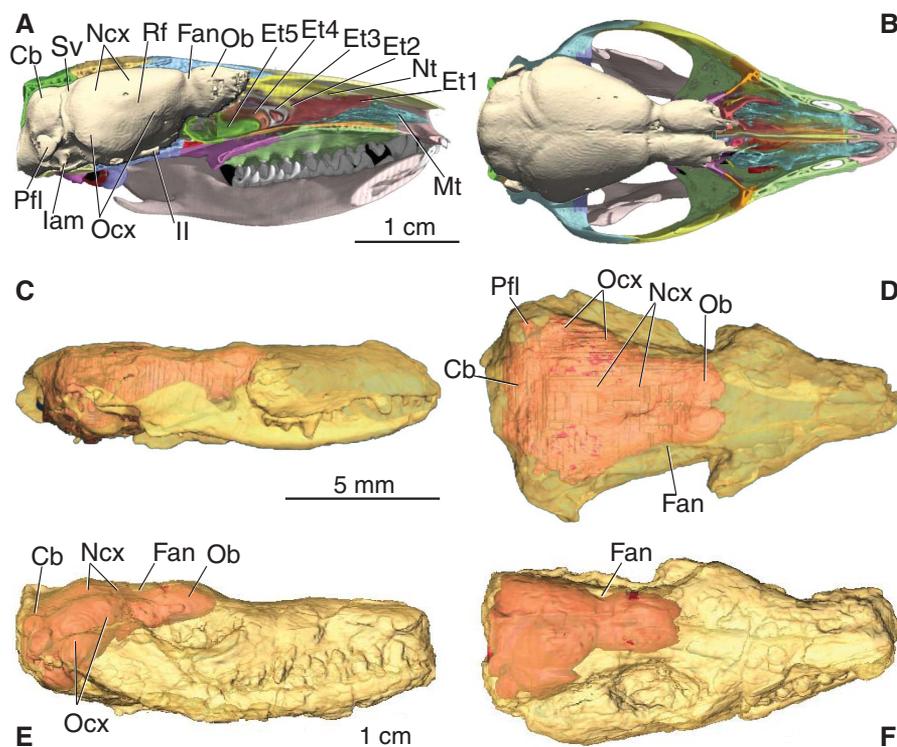
Fig. 1. HRXCT images of (**A** and **B**) *Monodelphis*, (**C** and **D**) *Hadrocodium*, and (**E** and **F**) *Morganucodon*, in lateral and dorsal views, with bone cutaway [(A) and (B)] and rendered translucent [(C) to (F)] to show endocasts. Cb, cerebellum; Et, endoturbinals 1 to 5; Fan, annular fissure; Iam, internal acoustic meatus; II, optic nerve; Mt, maxilloturbinal; Ncx, neocortex; Nt, nasoturbinal; Ob, olfactory bulb; Ocx, olfactory (pyriform) cortex; Pfl, paraflocculus; Rf, rhinal fissure; and Sv, venous sinus.

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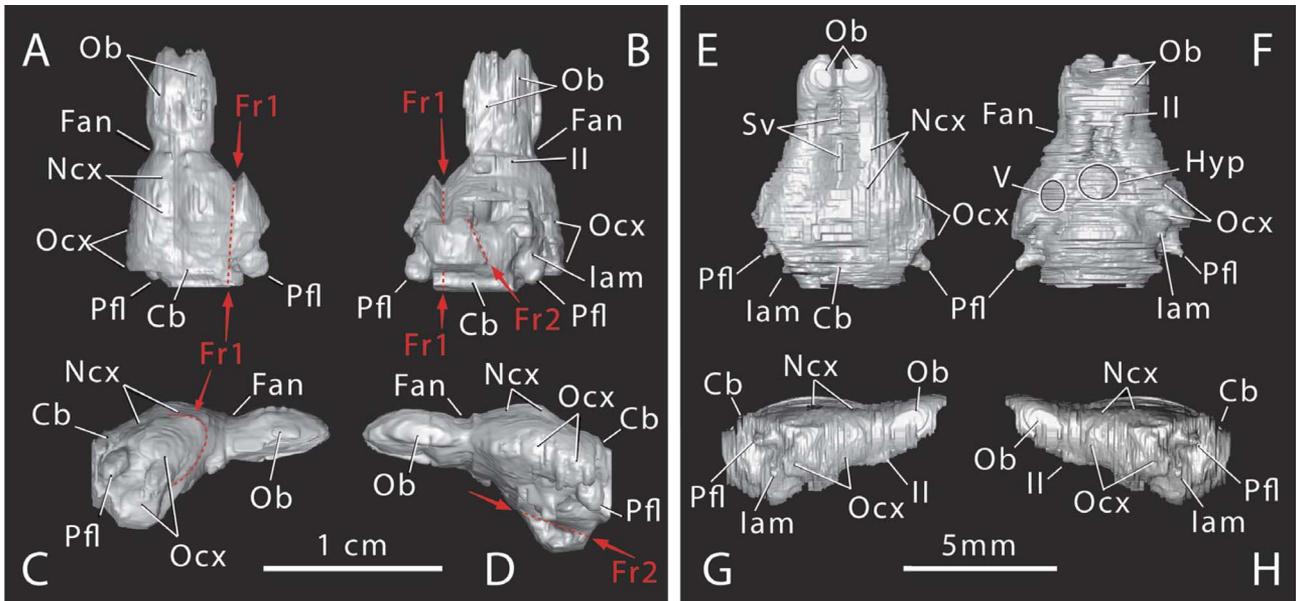
Fig. 2. Digital endocasts of (**A** to **D**) *Morganucodon* and (**E** to **H**) *Hadrocodium* in dorsal [(A) and (E)], ventral [(B) and (F)], right lateral [(C) and (G)] and left lateral [(D) and (H)] views. Cb, cerebellum; Fr1 and Fr2, postmortem fractures displacing parts of endocast; Fan, annular fissure; Hyp, hypophysis; Iam, internal acoustic meatus; II, optic nerve; Ncx, neocortex; Ob, olfactory bulb; Ocx, olfactory (pyriform) cortex; Pfl, paraflocculus; Sss, superior sagittal sinus; and V, trigeminal nerve.

stalk. The cerebellum is also enlarged, implying expansion of the basal nuclei, thalamus, and medulla, and the spinal cord is thicker. The brain now resembles living mammals more than basal cynodonts in shape and proportions.

Elaboration of the neocortex probably also contributed to encephalization in basal mammaliaformes. Dominating the neocortex is a single primary somatosensory field (*1*) that maps sensation from mechanoreceptors in the skin, hair follicles, muscle spindles, and joint receptors (Fig. 4A). Its conscious component involves tactile exploration and body surface monitoring (*3*). Peripheral somatosensory input is mapped to the neocortex as an "animunculus" (Fig. 4A). A parallel neocortical motor map contains pyramidal neurons that give rise to the pyramidal tract (Fig. 4B), which projects via the brainstem into the spinal column to program and execute skilled movements requiring precise control of distal musculature (*3*, *23–25*).

In living mammals, the boundary between neocortex and olfactory cortex is marked by the rhinal fissure. This structure is not visible on the endocast of *Morganucodon* or *Hadrocodium* and is faint (Fig. 1A) or invisible on endocasts in most small living mammals, although observable on the brain itself (*6*, *22*, *26*). However, another basal mammaliaform, *Castorocauda lutrasimilis* (*27*), preserves integumentary evidence suggesting that the neocortex was well developed. *Castorocauda* is a Middle Jurassic (~165 Ma) docodont (*27*), a clade first appearing in the Late Triassic and closely related to *Morganucodon* (*9–11*). *Castorocauda* is known from a flattened skeleton that preserves the oldest evidence of a thick pelt that covered the body. Both guard hairs and an underfur of vellus hairs left carbonized residues and physical impressions as thin grooves and traces.
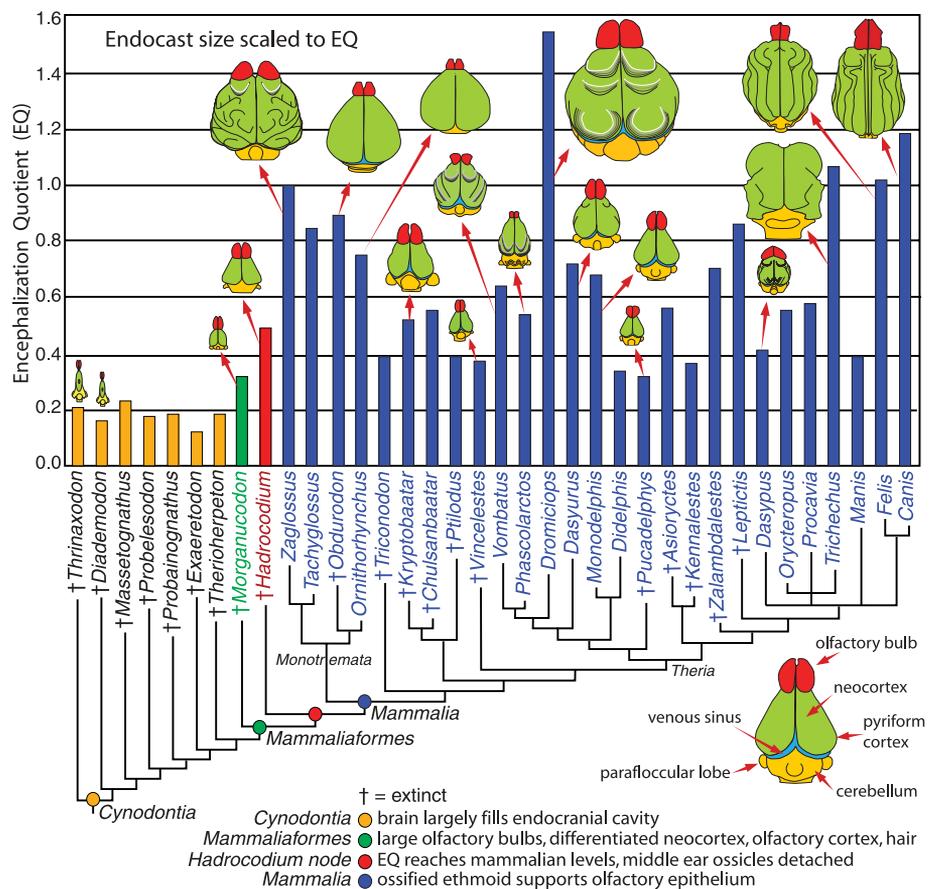
Fig. 3. Patterns of brain evolution in basal cynodonts and selected crown Mammalia. EQ is shown in bar chart; selected endocasts are scaled to EQ (*12*).

Body hair develops as migrating neural crest cells induce patterns of tiny placodes that mature into hair follicles equipped with mechanoreceptors (*25*). These include lanceolate endings (velocity detectors excited by hair deflection), Ruffini receptors (tension receptors activated as hair is bent), and Merkel cells (slowly adapting sensors) (Fig. 4C). In ontogeny, hair is first sensory, and
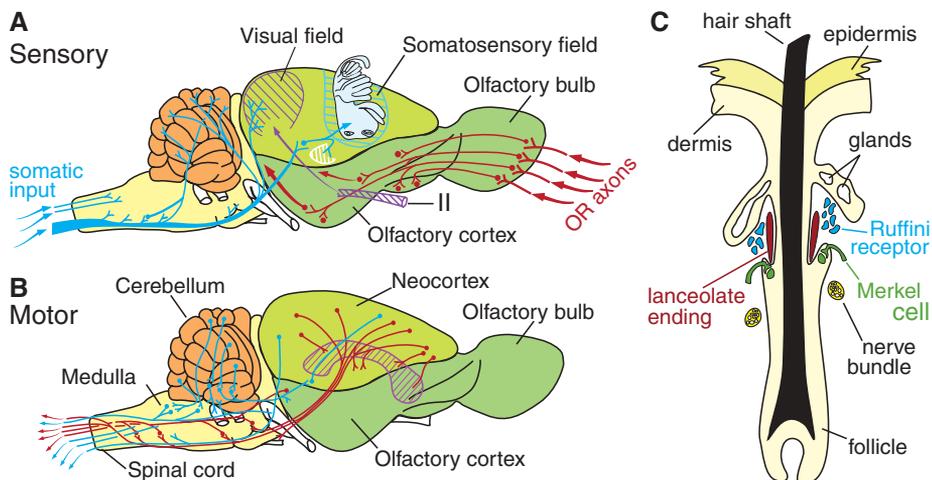
Fig. 4. Circuitry schematic of modern opossum (*Didelphis*) brain showing (**A**) sensory input and (**B**) motor outputs [modified after (*3*)]. (**C**) Schematic innervation of an opossum guard hair [modified after (*28*)].

only later does it insulate, as underfur thickens and thermoregulation matures (*28*). Tactile signals are transmitted to the primary somatosensory field, where their morphogenic action induces formation of the sensory and motor maps (*23–25*). The pelt in *Castorocauda*, in addition to the size and shape of the endocast in *Morganucodon*, implies that the neocortex differentiated early in mammaliaform history.

Increased sensitivity in olfaction, and improved tactile resolution and motor coordination account for much of the first pulse in pre-mammalian encephalization. Enhanced high-frequency hearing is also implicated. The middle ear ossicles are highly reduced (but still attached to the lower jaw), and the cochlea is now prolonged into a short, curved tube (*9*). Comparative neuroanatomy (*1*) suggests that neocortical expansion also supported an enhanced visual field (Fig. 4A), but bony correlates are lacking in these fossils.

Hadrocodium is the closest known extinct relative of crown Mammalia (*9, 11, 13*). It marks a second encephalization pulse, with an EQ of ~0.5 that lies within the mammalian range (Fig. 3). Expanded olfactory bulbs and olfactory cortex account for most of the increase. The middle ear ossicles are now detached from the jaw and suspended beneath the cranium, a condition otherwise confined to crown Mammalia (*10, 11, 13, 15*). Growth of the olfactory cortex in early ontogeny of the living didelphid *Monodelphis* separates the auditory ossicles from their primary (and ancestral) attachment to the mandible (*20, 21*) to develop the same anatomical relations seen in *Hadrocodium*. This famous transformation evidently had little effect on hearing performance because the size and complexity of the cochlea is no different than in *Morganucodon* (*9, 13, 22, 29*). The cerebellum in *Hadrocodium* bulges backward, bending the occipital plate into an arch that transmitted a thick spinal cord, implying enhanced motor-sensory integration.

The origin of crown Mammalia marks a third pulse of olfactory elaboration, as the ethmoid

turbinals ossify to form both the cribriform plate and a rigid scaffold in the nasal cavity for epithelium containing the odorant receptor (OR) neurons (*10, 15*). Activation of OR genes induces olfactory epithelial growth, in turn inducing turbinal growth and ossification (*30*). Ossified turbinals afford a 10-fold (or more) increase in olfactory epithelial surface within the nasal cavity. The maxilloturbinal also ossifies at this same time, affecting a sevenfold (or more) increase in respiratory epithelial surface (*30*). It functions in water balance, and its appearance in Mammalia ancestrally may reflect elevated metabolism.

Our data suggest that in basal mammaliaforms, a first pulse of encephalization was driven by increasing resolution in olfaction and tactile sensitivity and enhanced neuromuscular coordination. With a pelt, basal mammaliaforms were probably also endothermic, and the ontogeny of thermoregulation implies parental care (*28*). Endothermy may have been a consequence of encephalization because a large brain is metabolically expensive to maintain (*5*). However, metabolism is under hormonal regulation that does not command large cerebral regions, and thus did not itself drive encephalization (*3*). *Hadrocodium* records a second pulse of encephalization, probably also driven principally by olfaction.

The ancestral species of Mammalia amplified these inheritances in a third pulse of olfactory elaboration because its ossified ethmoid complex allowed full expression of its huge OR genome, which is an order of magnitude larger than in most other vertebrates (*31*). Only much later did acute visual and auditory systems evolve among mammals (*29*). In some descendents, the olfactory system was further elaborated, whereas in others it was reduced and supplanted by alternate sensory modalities, such as electroreception and sonar. But at its start, the brain in the ancestral mammal differed from even its closest extinct relatives specifically in its degree of high-resolution olfaction, as it exploited a world of information dominated to an unprecedented degree by odors and scents.

References and Notes

1. J. H. Kaas, in *Evolutionary Neuroscience*, J. H. Kaas, Ed. (Academic Press, New York, 2009), pp. 523–544.
2. F. Aboitiz, J. Montiel, *Adv. Anat. Embryol. Cel.* **193**, 1 (2007).
3. R. Nieuwenhuys, H. J. Ten Donkelaar, C. Nicholson, Eds., *The Central Nervous System of Vertebrates* (Springer-Verlag, Berlin, 1998).
4. R. G. Northcutt, J. H. Kaas, *Trends Neurosci.* **18**, 373 (1995).
5. J. Allman, *Neurosciences* **2**, 257 (1990).
6. H. J. Jerison, *Evolution of the Brain and Intelligence* (Academic Press, New York, 1973).
7. H. J. Jerison, in *Evolutionary Neuroscience*, J. H. Kaas, Ed. (Academic Press, New York, 2009), pp. 497–508.
8. T. B. Rowe, T. H. Rich, P. Vickers-Rich, M. Springer, M. O. Woodburne, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 1238 (2008).
9. Z. Kielan-Jaworowska, R. L. Cifelli, Z.-X. Luo, *Mammals from the Age of Dinosaurs: Origin, Evolution, and Structure* (Columbia Univ. Press, New York, 2004).
10. T. B. Rowe, *J. Vertebr. Paleontol.* **8**, 241 (1988).
11. Z.-X. Luo, *Nature* **450**, 1011 (2007).
12. Materials and methods are available as supporting material on *Science* Online.
13. Z.-X. Luo, A. W. Crompton, A.-L. Sun, *Science* **292**, 1535 (2001).
14. T. E. Macrini, dissertation, University of Texas, Austin, (2006).
15. J. A. Gauthier, A. G. Kluge, T. Rowe, *Cladistics* **4**, 105 (1988).
16. T. B. Rowe, W. Carlson, W. Bottorff, *Thrinaxodon: Digital Atlas of the Skull.* (Univ. of Texas Press, Austin, TX, CD-ROM, ed. 2, 1995).
17. J. A. Hopson, in *Biology of the Reptilia*, C. Gans, R. G. Northcutt, P. Ulinski, Eds. (Academic Press, New York, 1979), pp. 39–46.
18. J. C. Quiroga, *J. Hirnforsch.* **21**, 299 (1980).
19. J. C. Quiroga, *J. Hirnforsch.* **25**, 285 (1984).
20. T. S. Kemp, *J. Vertebr. Paleontol.* **29**, 1188 (2009).
21. T. B. Rowe, *Science* **273**, 651 (1996).
22. T. B. Rowe, *Cal. Acad. Sci. Memoir* **20**, 71 (1996).
23. K. C. Catania, in *Evolutionary Neuroscience*, J. H. Kaas, Ed. (Academic Press, New York, 2009), pp. 697–714.
24. L. Krubitzer, D. L. Hunt, in *Evolutionary Neuroscience*, J. H. Kaas, Ed. (Academic Press, New York, 2009), pp. 545–568.
25. J. Zelená, *Nerves and Mechanoreceptors: the Role of Innervation in the Development and Maintenance of Mammalian Mechanoreceptors* (Chapman & Hall, London, 1994).
26. T. E. Macrini, T. B. Rowe, J. L. VandeBerg, *J. Morphol.* **268**, 844 (2007).
27. Q. Ji, Z.-X. Luo, C.-X. Yuan, A. R. Tabrum, *Science* **311**, 1123 (2006).
28. A. J. Hulbert, in *The Developing Marsupial. Models for Biomedical Research*, C. H. Tyndale-Biscoe, P. A. Janssens, Eds. (Springer-Verlag, Berlin, 1988), pp. 148–161.
29. Z.-X. Luo, I. Ruf, J. A. Schultz, T. Martin, *Proc. Biol. Sci.* **278**, 28 (2011).
30. T. B. Rowe, T. P. Eiting, T. E. Macrini, R. A. Ketcham, *J. Mamm. Evol.* **12**, 303 (2005).
31. Y. Niimura, *Genome Biol. Evol.* **1**, 34 (2010).