

Virtual endocast of *Ignacius graybullianus* (Paromomyidae, Primates) and brain evolution in early primates

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Extant primates are distinctive among mammals in having relatively large brains. As stem primates, Paleogene plesiadapiforms provide direct information relevant to the earliest stages in the evolution of this characteristic. Here we describe a virtual endocast reconstructed from ultra high resolution X-ray computed tomography data for the paromomyid plesiadapiform *Ignacius graybullianus* (USNM 421608) from the early Eocene of Wyoming. This represents the most complete endocast known for a stem primate, allowing for an unprecedented study of both size and fine details of anatomy. Relative to fossil and extant euprimates, *I. graybullianus* had large olfactory lobes, but less caudal development of the cerebrum and a poorly demarcated temporal lobe, suggesting more emphasis on olfaction and a less well developed visual system. Although its brain was small compared to those of extant primates, the encephalization quotient of *I. graybullianus* is higher than that calculated for Paleocene *Plesiadapis cookei* and overlaps the lower portion of the range documented for fossil euprimates. Comparison to the basal gliroid *Rhombomylus* suggests that early primates exhibited some expansion of the cerebrum compared to their ancestors. The relatively small brain size of *I. graybullianus*, an arboreal frugivore, implies that neither arboreality nor frugivory was primarily responsible for the expanded brains of modern primates. However, the contrasts in features related to the visual system between *I. graybullianus* and fossil and extant euprimates suggest that improvements to these portions of the brain contributed to increases in brain size within Euprimates.

computed tomography | Eocene | plesiadapiforms | vertebrate paleontology | Wyoming

On average, extant primates have larger brains relative to body mass than other mammalian orders (1), and this feature is often listed among the order's diagnostic features (2). To determine whether large brain size evolved in the common ancestor of all primates, or in parallel in multiple primate lineages, it is necessary to study its evolutionary history. Several fairly complete endocasts have been described (3) for early euprimates (i.e., adapoids and omomyoids). These specimens have been controversial in their interpretation, with opinions differing on whether or not they provide evidence for expansion of the brain during the earliest phases of primate evolution (4, 5). An element missing from this debate has been a good record of endocasts from the primate stem. To date, only partial endocasts of 2 stem primates (plesiadapiforms) (Fig. 1) have been described, pertaining to *Megadelphus lundeliusi* (8) and *Plesiadapis cookei* (9). The development of ultra high resolution X-ray computed tomography techniques has made it possible to extract "virtual endocasts" from even relatively small, fragile specimens. For the current study, a virtual endocast was reconstructed from a nearly complete cranium of *Ignacius graybullianus* (USNM 421608) from the Early Eocene of Wyoming (10, 11). Although occurring relatively late in the evolutionary history of plesiadapiforms, this specimen represents the most complete endocast known for a stem primate, allowing for an

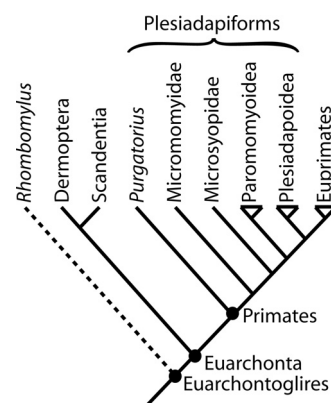


Fig. 1. Hypothesis of relationships for primates and other members of Euarchontoglires discussed in the text based on the results of Bloch et al. (6). This study did not include *Rhombomylus*, which is added here as the sister taxon to Euarchonta based on its hypothesized position as a basal gliroid (7). Key taxa discussed in the text include the plesiadapiforms *Megadelphus* (Microsyopidae), *Ignacius* (Paromomyoidea), and *Plesiadapis* (Plesiadapoidea). Euprimates refers to primates of modern aspect, including extant forms and fossil taxa (e.g., adapoids, omomyoids) that share critical traits such as the presence of a postorbital bar and nails on all their digits with living species.

unprecedented study of both size and fine details of anatomy. In the absence of comparable specimens from earlier occurring or more primitive stem primates, these data provide the only basis for assessing the timing of brain size increase in primate evolution, and for testing competing adaptive hypotheses of primate brain expansion using the fossil record.

Description and Comparisons. USNM 421608 is a nearly complete skull of *Ignacius graybullianus*, prepared by P. Houde by acid etching from a limestone nodule (10–12) from Houde site 24 (near University of Michigan locality SC-125), which is in the *Cardiophorus radinskyi* range zone of the Wasatchian (11), making it between 54 and 55 million years old (13). Detailed descriptions of this specimen have been published previously (10, 11, 14, 15). Of relevance to the endocast reconstruction, it is worth noting that the specimen is slightly crushed dorsoventrally, which implies that volume measures should be viewed as minimum estimates. This distortion also influences some aspects of the shape of the endocast, as discussed below.

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Table 1. Measurements of the endocast of *Ignacius graybullianus*

Measurement	<i>Ignacius graybullianus</i>	<i>Plesiadapis cookei</i>
Total length	30.79	42
Maximum width	19.44	22
Maximum depth	12.15	≈12–13
Olfactory bulb length	6.28	10
Olfactory bulb length/total length	0.20	0.24
Ratio of olfactory bulb length to the length of rest of the brain	1:3.9	1:3.2
Olfactory bulb width	3.935	5
Foramen magnum height	5.15	8.5
Foramen magnum width	7.31	6
Endocast width/length ratio	0.63	0.52
Endocast height/length ratio	0.39	0.30
Endocast height/width ratio	0.63	0.57
Total endocast volume	2.14	5
Olfactory bulb volume	0.12	
Percentage of the volume of the brain composed of the olfactory bulbs	5.53	
Right optic nerve cross-sectional area*	1.92	
Left optic nerve cross-sectional area*	1.66	
Average optic nerve cross-sectional area	1.79	

Lengths are in millimeters, areas in millimeters squared, volumes in cubic centimeters, and mass in grams. Data for *P. cookei* (9) are included for comparison.

*Calculated from the optic nerve roots on the endocast as the product of the maximum diameter and the diameter perpendicular to the maximum

As in *P. cookei*, the endocast of *I. graybullianus* has large olfactory bulbs relative to the overall volume of the brain for a primate, although smaller than in some fossil eutherians [e.g., *Leptictis* (16), an “insectivore-grade” mammal often considered relatively primitive among eutherians and *Asioryctes* (17), a primitive eutherian from the Cretaceous of Mongolia] [supporting information (SI) Fig. S1 and Table S1]. The bulbs comprise the rostralmost 20% of the length of the endocast and 5.5% of its total volume (Table 1), and are located between the orbits, which contributes to the broad interorbital distance (11) (Fig. 2). There is a distinct circular fissure separating the olfactory bulbs from the cerebrum (Fig. 3A), implying that the cerebrum did not overlap onto the olfactory bulbs. While this contrasts with the condition in modern primates, this lack of overlap is seen in fossil omomyoids and adapoids who also have pedunculated olfactory bulbs (3). Clearly, expansion of the frontal lobes in primates occurred after the early Tertiary (18). In contrast to modern primates and fossil omomyoids, but similar to fossil adapoids, the caudal aspect of the cerebrum of *I. graybullianus* does not overlap with the cerebellum. Unlike any documented extant or fossil euprimate, a portion of the midbrain between the caudal aspect of the cerebrum and rostral aspect of the cerebellum is exposed. Two distinct swellings, interpreted as the caudal (inferior; auditory) colliculi (9) in the endocast of *P. cookei*, are also present in that of *I. graybullianus*. Alternatively, it is possible that the swellings represent the rostral (superior; visual) colliculi, as they are exposed in this position in newborn *Tupaia* (19). However, in *Tupaia* this is a product of the very large size of the rostral colliculi, associated with specializations for visual processing that *I. graybullianus* seems to lack. Therefore, an identification of these structures as the caudal colliculi is more likely. The meaning of the exposure of the colliculi is unclear. Gingerich and

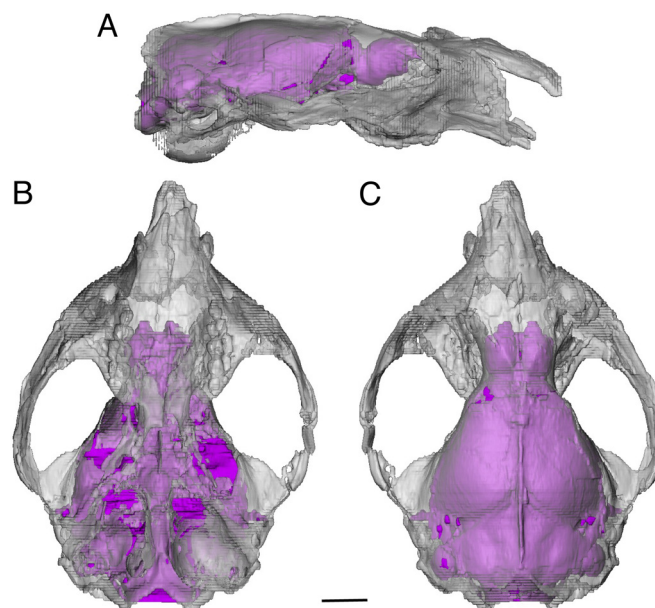


Fig. 2. Endocast of *Ignacius graybullianus* (USNM 421608) inside a translucent rendering of the cranium in (A) lateral, (B) ventral, and (C) dorsal views. (Scale bar, 5 mm.)

Gunnell (9) suggested that the “relative size of the quadrigeminal colliculi suggests that acoustic reflexes were better developed than visual reflexes in *P. cookei*.” In light of the fact that the rostral colliculi are not exposed on the endocast of either *I. graybullianus* or *P. cookei*, we are unable to speculate on their relative size or on the degree of development of the related reflexes. While exposure of the midbrain may be a primitive characteristic, it has also been argued that this feature may evolve secondarily as a result of sensory specialization (20). Such a claim has been made, for example, for the exposure of the colliculi in dermopterans (9, 20). Despite these uncertainties, it is clear that the cerebra of both *I. graybullianus* and *P. cookei* show less caudal development than seen in either fossil or extant euprimates. In contrast, the midbrain is less broadly exposed in *Megadelphus lundeliusi*, and no colliculi are visible on the endocast (8).

The endocast of the cerebrum of *I. graybullianus* is almost smooth, without sulci (with the exception of the rhinal fissure; see below), in contrast to that of *P. cookei* and *M. lundeliusi*. While this might be interpreted as a product of a lack of cerebral expansion, the lack of sulci in small-brained modern mammals (21) implies sulci would not necessarily be expected on an endocast of an animal as small as *I. graybullianus*.

There is a notable difference in the degree of development of the temporal lobes between *I. graybullianus* and both extant and fossil euprimates. No clear sylvian fissure is present in the endocast of *I. graybullianus* (the relevant region is not preserved for either *P. cookei* or *M. lundeliusi*); rather, a slight indentation is present that is more comparable to the sylvian fossa identified in *Tupaia* (1, 22) than to the more distinct fissure of most euprimates. This is suggestive of less development of the temporal lobe in *I. graybullianus*, although it is worth noting that one fossil euprimate [*Smilodectes* (23)] similarly lacks a clear sylvian fissure. Even relative to that of *Smilodectes*, however, the endocast of *I. graybullianus* is less expansive ventrally in the caudal portion of the cerebrum and lacks a clear temporal pole. It could be argued that this impression is accentuated by the somewhat flattened nature of the specimen. However, while this flattening would be expected to expand the mediolateral dimension, the endocast of *I. graybullianus* is narrower mediolaterally in this

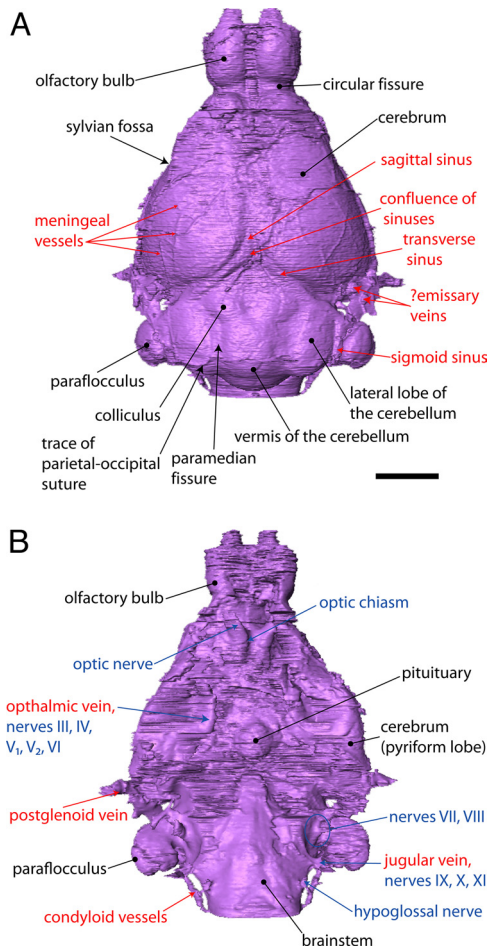


Fig. 3. Labeled endocast of *Ignaciuss graybullianus* (USNM 421608) in (A) dorsal and (B) ventral views. (Scale bar, 5 mm.)

region than in fossil euprimates. For example, the most lateral point of the cerebral hemispheres is medial to the lateral extent of the paraflocculus, a characteristic considered primitive for therians (21). Although the paraflocculus is not well preserved in most early Tertiary euprimate endocasts, in *Adapis parisiensis* the cerebrum appears to exhibit the same condition as modern euprimates, extending laterally beyond the level of the floccular casts (24).

A key landmark of relevance to assessing the degree of cerebralization in *I. graybullianus* is the position of the rhinal fissure that marks the division between the paleocortex and neocortex. The likely location of this structure is indicated by a vascular canal on the lateral surface of the endocast (Fig. 4) that extends between the orbit and the postglenoid vein. The rhinal fissure shares a close relationship with this channel in modern lemuriiforms, and this structure has been interpreted as a landmark for the fissure in both fossil primates (1, 3, 23) and nonprimates [e.g., *Leptictis* (16)]. Although the rhinal fissure is located further ventrally than in some living lipotyphlan insectivores (e.g., *Solenodon*, *Erinaceus*), its position on the lateral surface of the cerebrum is more similar to extant tree shrews than to euprimates. Even in the endocasts of early Tertiary fossil euprimates, the rhinal fissure is located nearer to the apex of the temporal pole of the brain than in *I. graybullianus* (1, 3), implying greater development of the neocortex. However, the vascular channel in *I. graybullianus* is located much further ventrally than the structure tentatively identified as a rhinal fissure for *P. cookei* (9). This apparent difference may be a product of less complete

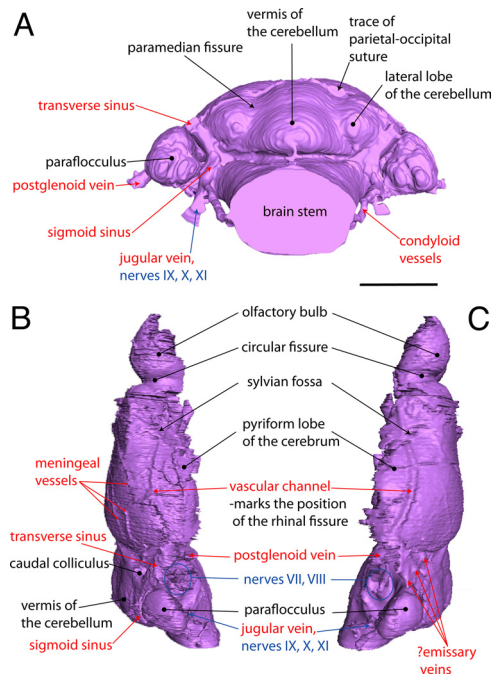


Fig. 4. Labeled endocast of *Ignaciuss graybullianus* (USNM 421608) in (A) caudal, (B) right lateral, and (C) left lateral views. (Scale bar, 5 mm.)

preservation of the cranium in *P. cookei*. The structure identified (9) as a rhinal fissure in the endocast of *P. cookei* is near the edge of the region that is preserved, and there is not enough of the endocranial surface preserved laterally to assess whether or not a vascular channel was present more ventrally, as seen in *I. graybullianus*. The supposed rhinal fissure of *P. cookei* is also in approximately the same position as the suprasylvian sulcus of *M. lundeliusi* (8), so it may alternatively represent that feature. Szalay (8) mentioned the presence of a vascular canal on the lateral side of the endocast of *M. lundeliusi*, but does not indicate its relative position ventrally; the latex endocast Szalay (8) described is no longer available for study. (The original latex endocast was located in the collection of the American Museum of Natural History, but appears to have melted, so that it no longer retains any of the described morphology.)

The endocast of *I. graybullianus* preserves a well-developed cerebellum, similar in relative size to those of *A. parisiensis* and *Smilodectes*. The relative size of this region is difficult to assess in omomyoids because it is tucked under the cerebrum. Unlike the endocast of *Smilodectes*, the fissura prima is not evident on the endocast of *I. graybullianus*. A longitudinal “crease” running across the cerebellum that might be confused with this fissure sits at the level of the parietal-occipital suture, and so is more plausibly interpreted as a trace of that suture (see Fig. 3A). Well-demarcated paramedian fissures are evident, however, separating the lateral lobes of the cerebellum from the vermis. This configuration is characteristic of not only extant and fossil euprimates but of therians more generally (21). The caudal end of the cerebellum is well separated from the brainstem in the endocast of *I. graybullianus*, presumably because the tentorium cerebelli was ossified (11) (see Fig. 4).

The endocast of *I. graybullianus* preserves casts of several elements of the venous drainage system, including channels for the sagittal, transverse, and sigmoid sinuses, and postglenoid vein. Although these structures are fairly typically arrayed for a mammal, the presence of a substantial canal for the postglenoid vein is markedly different from dermopterans that lack this vessel (25). Casts of the parietal foramina are visible on the

Table 2. Encephalization quotient estimates for *Ignacius graybullianus*

Source of body mass estimate	Body mass	EQ [Jerison (16)]	EQ [Eisenberg (27)]
Cranial length insectivore equation (29)	231	0.47	0.69
Cranial length horizontal primate PGLS equation (30)	253 (140–460)	0.44 (0.29–0.65)	0.65 (0.42–1.0)
Cranial length generic primate equation (1)	286	0.40	0.59
Upper molar area (31)	375 (306–460)	0.34 (0.29–0.39)	0.48 (0.42–0.56)

Body mass estimates are in grams. Values in brackets represent 95% confidence intervals for body mass and the EQ estimates that correspond with the endpoints of the body mass confidence intervals. The insectivore equation was based on a least-squares regression analysis of a sample of 64 species of extant insectivorous mammals (29); although *I. graybullianus* was likely not predominantly insectivorous, this estimate was included because the estimate from this equation for *P. cookei* was compatible with that from its postcranium (9), which suggests that this equation may reflect scaling relationships in plesiadapiforms. The “generic primate equation” was calculated based on the major axis for a sample of 36 modern primate species, including forms with both vertical and horizontal habitual body positions (1). The horizontal primate PGLS equation was calculated from a phylogenetic least-squares analysis of 22 species of living primates who habitually exhibit horizontal body positions (21).

endocast. These may represent openings for emissary veins (see Figs. 3A and 4), an interpretation supported by their apparent continuity with the vascular canal discussed above. The cast of the sigmoid sinus can be traced to the cast of a single jugular foramen on the caudal aspect of the endocast (see Fig. 4), that presumably transmitted cranial nerves IX, X, and XI, as well as the internal jugular vein. The cast of the sigmoid sinus is also continuous with the well-demarcated channel for the condyloid vessels (see Fig. 4).

The ventral surface of the endocast of *I. graybullianus* preserves a cast of the hypophyseal fossa. This fossa was slightly wider than long (width, 3.0 mm; length, 2.7 mm), but significantly longer than deep (depth, ≈2.0 mm). In mammals the pituitary gland sits upon rather than within the hypophyseal fossa (26). Thus, the hypophyseal fossa is a poor representative of the size of the pituitary gland and the broader evolutionary significance of its dimensions are unclear (21). Casts of the foramina for most of the other cranial nerves can be seen on the ventral surface of the endocast (Fig. 3B). Rostral to the hypophyseal fossa, the casts of the optic chiasm and the nerve roots of the optic nerves are present. This is of particular interest because no measurements have ever been reported of the optic foramina in *I. graybullianus*, so the diameters of these casts (see Table 1) represent the first proxy available for the size of the optic nerve. This is relevant to assessing activity period in *I. graybullianus* (see *SI Text* and below). Lateral to the hypophyseal fossa are casts of the sphenorbital fissure. *I. graybullianus* did not possess a separate foramen rotundum (11), so this fissure presumably transmitted the maxillary branch of the trigeminal nerve (V_2) in addition to the ophthalmic vein and cranial nerves III, IV, V_1 , and VI. The lack of a foramen rotundum is a contrast between *I. graybullianus* and other plesiadapiforms, scandentians, and some euprimates (27, 28). Casts are present for the internal auditory meatus (for cranial nerves VII, VIII) just ventral to the paraflocculus, and for the hypoglossal foramen (for cranial nerve XII) on the brainstem. The basicranium is not well enough preserved to allow for identification of the anterior carotid foramen or foramen ovale (if present) on the endocast. There are no clear indications of divisions of the brainstem; for example, the pons is not distinct.

Brain Size and Encephalization Quotient. The endocranial volume of USNM 421608 is 2.14 cc (see Table 1). To avoid issues of intraspecific variation, estimates of body mass were made only on upper molar area and cranial length, dimensions that could be estimated for the specimen in question (Table 2). These estimates (including confidence intervals) range from 140 to 460 g. There are multiple equations that have been used to calculate the encephalization quotient (EQ). Using Jerison’s equation (18), the EQ of *I. graybullianus* ranges from 0.29 to 0.65, depending on the body mass estimate used, while Eisenberg’s equation (32) produces a range of EQs from 0.42 to 1.0. Even the lowest values

in these ranges are somewhat higher than the EQ estimates for *P. cookei* [EQ (Jerison) = 0.24; EQ (Eisenberg) = 0.31, calculated from a body mass estimate of 2,200 g] (Table S2). Gingerich and Gunnell (9) interpreted the values for *P. cookei* as implausibly low for a primate. However, we note that they limited their comparisons to extant primates. It has long been known that there is a temporal effect in brain size (18), making comparisons with other early Tertiary mammals more appropriate. Encephalization quotients for fossil euprimates extend significantly below the range for modern members of the order (Fig. 5). Indeed, the fossil euprimate *Pronycticebus gaudryi* has an EQ only slightly higher [0.34, Jerison’s formula (18); 0.45, Eisenberg’s formula (32)] than the lowest EQ estimates for *I. graybullianus* [see Table 2; body mass and cranial capacity estimates for *P. gaudryi* from Martin (1), see Table S2]. This would seem to suggest that the relative brain size for the plesiadapiforms is in keeping with what might be expected for a stem primate.

A more difficult question to answer is whether or not plesiadapiforms show any evidence of brain size expansion relative to their ancestors: —in other words, is brain expansion a feature that evolved at the base of the order Primates? Relative to a diverse array of “archaic” mammals from the late Cretaceous and early Tertiary (see Fig. 5 and Table S2), *I. graybullianus* (but not *P. cookei*) appears to have a higher than average EQ. The

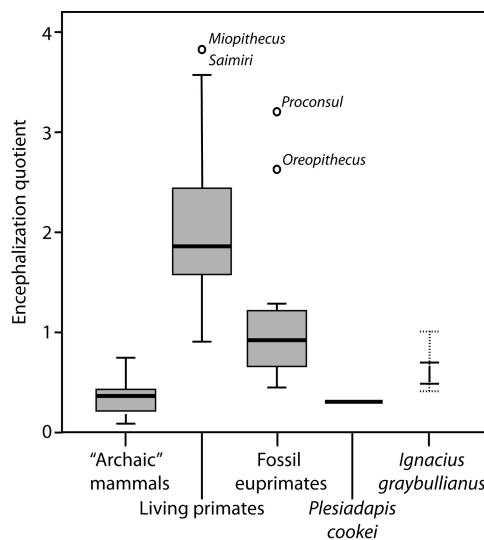


Fig. 5. Box plot of EQs, based on Eisenberg’s equation (32), of archaic mammals, extant and fossil nonhominin euprimates, and plesiadapiforms, calculated from intracranial volume. The archaic mammals include a variety of late Cretaceous and early Tertiary taxa. The range of estimates for *I. graybullianus* reflects the array of body mass estimates (including confidence intervals) given in Table 2. (See Table S2 for data.)

import of this finding is tempered, however, by EQs calculated for some primitive eutherians, which lie within the range of estimates for *I. graybullianus* [e.g., *Asioryctes*, EQ = 0.56 (Eisenberg) (32)], although this is in part because of their larger olfactory bulbs (see Fig. S1).

A more relevant comparison would be to close relatives of primates, such as other euarchontans. Both *I. graybullianus* and *P. cookei* have lower EQs than extant euarchontans (9). However, because early Tertiary mammals generally have lower cranial capacities than modern forms (18), it would be more appropriate to compare the plesiadapiforms to fossil tree shrews or dermopterans. Unfortunately, adequate fossil material has yet to be recovered for either of these groups. In light of the inferred close relationship between euarchontans and gliroids (33), the most relevant comparison available is to the basal gliroid *Rhombomylus*. Meng et al. (7) published illustrations of several endocasts of this early Eocene taxon, although they did not include any quantitative measures. Nonetheless, the endocasts of *Rhombomylus* appear more primitive [i.e., similar to *Asioryctes* (17)] than *I. graybullianus* in several features, including their short, caudally divergent cerebral hemispheres, and very broad exposure of the midbrain. Therefore, to the extent that this comparison is relevant, it suggests some modest brain expansion may have occurred at the Primates or Euarchonta node. What can be stated with more confidence is that the earliest primates had smaller brains than those of modern primates, implying that significant brain size increase must have occurred subsequently in primate evolution.

Hypotheses for Brain Size Increase in Primates. A number of factors have been suggested as potentially contributing to brain size increase in primates. One such factor is arboreality;—moving into the spatially complex 3-dimensional environment of the trees might have necessitated more processing power as well as visual and sensomotory specializations (34). A relationship between arboreality and brain size exists in small mammals (35), but not within Primates (36). Another factor is diet. Within Primates, frugivores typically have relatively larger brains than folivores (36), but the causes of this relationship are in debate. One explanation is that frugivory may be a more complex feeding strategy than folivory because food sources are more clumped and less consistently available, so that primates need a better memory and more processing power to successfully exploit fruit than leaves (36). Alternatively, a high-energy diet may be a necessary precursor to brain size increase (37). Barton and colleagues (38, 39) and Kirk (40) have argued that brain size increase in primates is strongly associated with evolution of the visual system, and that orbital convergence in particular is tied to increases in the size of visual brain structures. Frugivory may be related to this because diurnal frugivores show expansions to the visual system (38). This raises another factor of potential import to primate brain size evolution: activity period. Barton et al. (38) suggested that there were evolutionary trade-offs in primate evolution between diet and activity period, so that nocturnal primates seem to have larger olfactory bulbs, while diurnal primates have better developed visual systems, at least within frugivores. Finally, as part of the social brain hypothesis, social complexity and its proxy group size (37, 41) have been implicated in primate brain size evolution.

To assess the relevance of the endocast evidence from *I. graybullianus* to addressing these hypotheses, it is necessary to reconstruct as many of its ecological characteristics as possible. Beard (42) discussed a number of postcranial elements that he attributed to this species. He considered paromomyids to be gliding mammals, similar to extant dermopterans. Analysis of skeletal material of a close relative of *I. graybullianus*, *I. clarkforkensis*, has effectively refuted the gliding hypothesis, and indicated that paromomyids are better reconstructed as “cal-

litrichine-like” arboreal animals (6). In terms of diet, the paromomyid *Phenacolemur* has been suggested to have been broadly omnivorous based on similarities in its dentition to living *Petaurus* (43), or to have specialized on fruit, nectar, and gum (44). Relative to *Phenacolemur*, *Ignacius* has even lower crowned molar teeth, a feature associated with frugivory (45). Based on its combination of small orbit size and an apparently quite large optic nerve, *I. graybullianus* can be reconstructed as having been diurnal (see *SI Text* and Figs. S2 and S3). Unfortunately, there are no clear proxies for social complexity or group size in the skeleton.

The differences in the endocast between *Rhombomylus* and *I. graybullianus* may be in part a result of the latter’s arboreal habitus, although if this is the case it would be expected that these changes might have evolved at the Euarchonta node rather than the Primates node, as the common ancestor of Euarchonta was likely arboreal (46). Unfortunately, until well-preserved cranial fossils of scandentians and dermopterans are discovered, the timing of these changes cannot be assessed. The larger relative brain size of *I. graybullianus* compared to *P. cookei* may be attributable to a more frugivorous diet, because the latter has been reconstructed as quite folivorous (47). It is worthy of note, however, that *I. graybullianus*, an arboreal, frugivorous animal, has a brain smaller than that of an extant primate. This suggests that these factors are not adequate in and of themselves to produce the relative brain size expansion seen in extant forms. These findings would be consistent with the view that a high-energy diet may be required for the development of increased brain size, and not with the view that a big brain is required to effectively forage for fruit.

Most of the differences between the endocast of *I. graybullianus* and those of fossil and extant euprimates seem to relate to differences in the visual system, supporting the idea that improvements to vision were important in contributing to brain size increase in Euprimates. *I. graybullianus* has relatively divergent orbits and a small brain, which is consistent with the view that orbital convergence and the size of visual structures in the brain are coordinated in primate brain size evolution (39). The size of the optic foramen is a direct proxy for the amount of visual input received by the brain. A close relationship exists between the relative endocranial volume and relative optic foramen area in euprimates (40). As in euprimates, a relatively small optic nerve (based on the area of the stem of the optic nerve on the endocast) is associated with a low relative endocranial volume in *I. graybullianus*, supporting the idea that the small brain in this taxon is in part attributable to a less well developed visual system (Fig. S4). Interestingly, *I. graybullianus* seems to combine three characteristics that are generally not seen together in living primates (38):—large olfactory bulbs, diurnality, and frugivory. This highlights that fact that no living animal is a perfect analogue for *I. graybullianus*.

Conclusions

The endocast of *Ignacius graybullianus* is unique in providing detailed anatomical information on the brain for a stem primate, and allows the assessment of hypotheses about the early phases of primate brain expansion. Although there is some weak evidence that the earliest primates may have exhibited advances in the brain over their forbears, much of the brain expansion that characterizes modern primates must have occurred at later stages in the evolution of the order, at least in part in association with improvements to the visual system. The fact that *I. graybullianus* was able to function as an arboreal frugivore with a brain size smaller than that of a typical modern mammal suggests that these factors were not the critical elements contributing to the larger than average brain size of modern primates.

Methods

As detailed by Silcox (15), USNM 421608 was scanned with the OMNI-X Industrial Scanner at the Center for Quantitative Imaging, Pennsylvania State University. A total of 1,281 slices were reconstructed as 16-bit tiffs at a matrix size of $1,024 \times 1,024$, an interslice spacing of 0.03753 mm, and a pixel size of 0.03435 mm. The images were initially cropped to $1,024 \times 512$ using ImageJ (48) to remove blank areas. Each slice was manually segmented in ImageJ (48), and the 821 slices containing the braincase were stacked and converted to raw data using strip2raw, a DOS program developed by N. Jeffrey (University College London). The segmented images were opened in Amira 3.1.1 (Visage Imaging), and a surface rendering of the endocast was produced (see Figs. 3 and 4) using the labelfield module. To produce the images with the translucent cranium (see Fig. 2), every third slice from the full dataset (including segmented slices) was stacked in strip2raw and opened in Amira 3.1.1 (Visage Imaging). The cranial bone and endocast were selected in separate labelfield modules, to which different colors and transparencies were applied. Measurements were made in Amira 3.1.1 (Visage Imaging), or (when possible)

using calipers on the actual specimen. Body mass was estimated based on measurements of cranial length and M1 area from USNM 421608 using published regressions (1, 29–31). Intracranial volume was calculated in Amira from the reconstructed endocast and checked by calculating the area of the segmented region for each slice in ImageJ (48), multiplying this by the slice thickness, and then summing the result for each slice for either the full endocast, or for the olfactory bulbs alone. All graphs were produced using SPSS version 12.0 for Windows.

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