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Evolutionary Development of the Middle Ear in Mesozoic Therian Mammals
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significant internal heating and could differentiate if it formed before the decay of ^{26}Al. Indeed, if Pallas and Ceres did form contemporaneously with similar initial composition, Pallas could retain less heat and water because of its smaller size. Our fit shape is close to that of a hydrostatically relaxed spheroid, which for a mean density of 2400 kg/m^3 would have a and c axes of 283.4 and 251.6 km, respectively, at Pallas's current spin period. These dimensions correspond to the undifferentiated case; our uncertainties would not allow any discrimination of the few-kilometer difference reasonable differentiated models would make. In either case, Pallas's density and spectral properties are suggestive of a body in which water has played a key role. These new measurements of Pallas's size, shape, and reflectance build the case that it joins Ceres and Vesta as the third intact protoplanet: an evolved body with planet-like properties.

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Evolutionary Development of the Middle Ear in Mesozoic Therian Mammals

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The definitive mammalian middle ear (DMME) is defined by the loss of embryonic Meckel's cartilage and disconnection of the middle ear from the mandible in adults. It is a major feature distinguishing living mammals from nonmammalian vertebrates. We report a Cretaceous trechnotherian mammal with an ossified Meckel's cartilage in the adult, showing that homoplastic evolution of the DMME occurred in derived therian mammals, besides the known cases of eutriconodonts. The mandible with ossified Meckel's cartilage appears to be paedomorphic. Reabsorption of embryonic Meckel's cartilage to disconnect the ear ossicles from the mandible is patterned by a network of genes and signaling pathways. This fossil suggests that developmental heterochrony and gene patterning are major mechanisms in homplastic evolution of the DMME.

Stem therian mammals of the Mesozoic are relatives to modern marsupials and placentals that make up 99% of living mammals today. The marsupial-placental clade, also known as living Theria, is successively nested within the boreosphenidan mammals, the pretribosphenic mammals, and trechnotherians (*1*–*6*). The trechnotherian clade of living therians and spalacotheroids is one of 20 or so Mesozoic mammaliaform clades (*1*, *5*).

Spalacotheroids are a basal group of the trechnotherian clade (*6*–*10*), characterized by the acute triangulation of the molar cusp pattern, a precursor condition to the tribosphenic molars of the common ancestor to marsupials and placentals (*1*–*10*). Spalacotheroids have ancestral skeletal features of living therians (*9*, *10*). Here, we describe an Early Cretaceous spalacotheroid (Figs. 1 and 2) that sheds light on the evolution of the definitive mammalian middle ear (DMME) (*11*). Whether this key mammalian feature had a singular origin or had evolved multiple times is still being debated (*11*–*16*).

Maotherium asiaticus sp. nov. (*17*) shows a diagnostic pattern of main molar cusps arranged in an almost symmetric triangle, thus also known as symmetrodont molars. The postcanines show an increasingly acute (smaller) angle between cusps B′-A-C from the premolar toward the more posterior molars, a gradient in all symmetrodont mammals but most prominently developed in spalacotheroids (*6*–*10*). The upper cusps B′ and C are slightly conical, more closely resembling those of zhangheotheriids than those of other spalacotheroids. A wear facet is developed along the preparacrista (prevallum) between cusp A (paracone) and cusp B′ on M1 and M2, which erupted first in the molar series. However, the facet is not yet developed on the more posterior molars that would erupt later (*6*, *7*, *9*). Thus, the match of upper and lower wear facets occurred after eruption and after substantial occlusal contact of the upper-lower molars. The triangulated shearing surfaces on the molars suggest that *M. asiaticus* was an insectivorous mammal.

M. asiaticus was a generalized terrestrial mammal. It represented a common ecomorphotype and lifestyle among a wide range of ecomorphotypes of Mesozoic mammals (*18*–*21*). *M. asiaticus* is estimated to have a total body length from 150 mm to 155 mm and to weigh between 72 (scaling from its 28.5-mm mandibular length) and 83 g (scaling from skull length of 36.5 mm) [details in (*22*)]. In the hind-foot digit ray III of *M. asiaticus* and *M. sinensis*, the intermediate phalanx is short relative to the proximal phalanx; the proximal and intermediate phalanges are short relative to the metatarsal. Both suggest a terrestrial habit for *Maotherium*. That *M. sinensis* is a terrestrial mammal is also shown by its manual terminal phalanx shape and by phalangeal ratio that can be correlated with the terrestrial ecomorphotypes of extant mammals (*23*). *Zhangheotherium* and *Maotherium* are basal among spalacotheroids, and both had terrestrial habits (*9*, *10*), suggesting that spalacotheroids ancestrally were terrestrial with generalized locomotory function (*9*, *10*, *24*).

M. asiaticus has an ossified Meckel's cartilage. The cartilage has a compressed and tapering anterior limb that is solidly lodged in the

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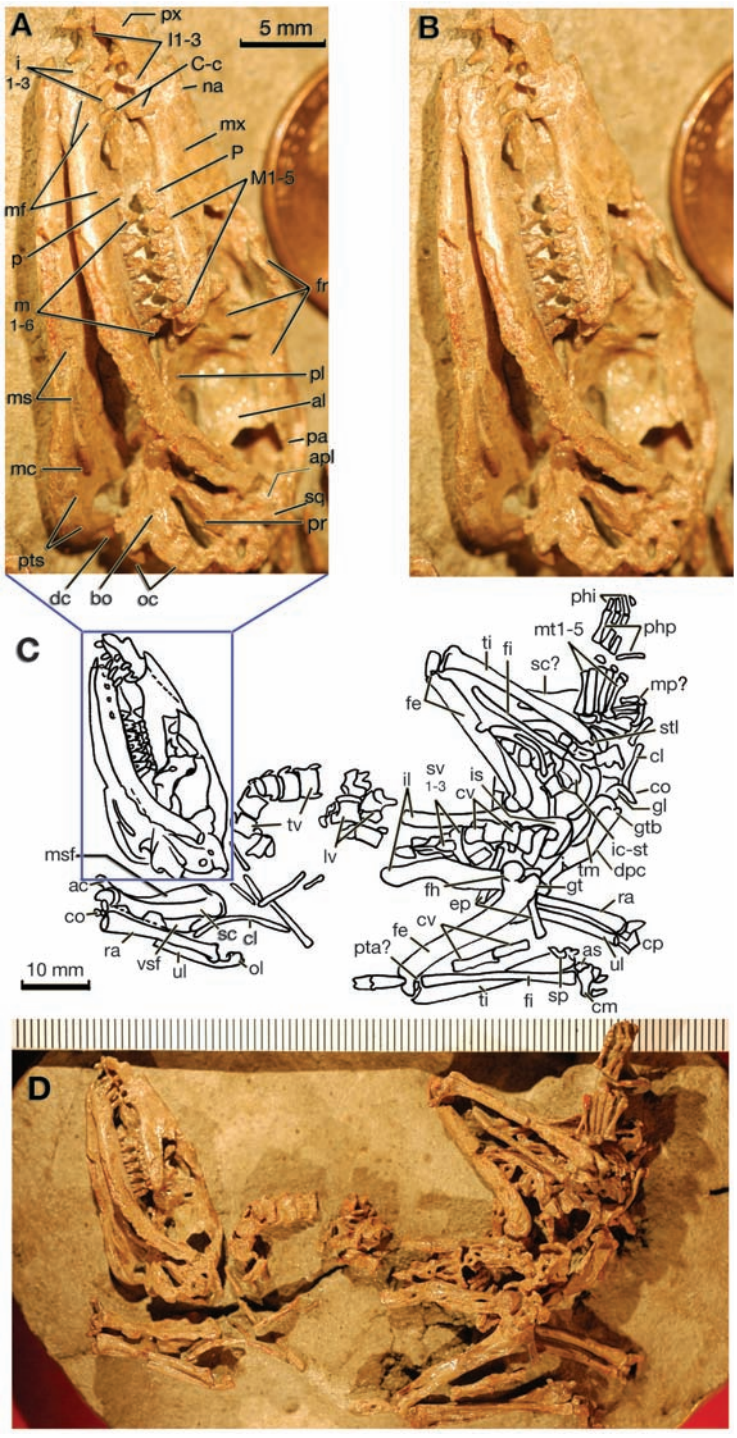

Fig. 1. Skeleton and skull of the Cretaceous spalacotheroid therian mammal *Maotherium asiaticus* [Henan Geological Museum (HGM) 41H-III-0321; holotype]. (**A** and **B**) Stereophotos of the skull with the ossified and in situ Meckel's cartilage connected to the mandible. (**C** and **D**) Skeletal feature identification and specimen photo. Abbreviations are as follows: ac, acromion; al, alisphenoid; apl, anterior lamina of petrosal; as, astragalus; bo, basioccipital; C and c, upper and lower canines; cl, clavicle; cm, calcaneum; co, coracoid process (scapula); cp, carpals; cv, caudal vertebrae; dc, dentary condyle; dpc, deltopectoral crest; ep, epipubis; fe, femur; fh, femoral head; fi, fibula; fr, frontal; gl, glenoid of scapula; gt, greater trochanter of femur; gtb, greater tubercle of humerus; I and i, incisors; ic, interclavicle; il, ilium; is, ischium; lv, lumbar vertebrae; M and m, upper and lower molars; mc, Meckel's cartilage; mf, mental foramina; msf, medial scapular facet; mp?, metacarpals?; mt, metatarsal; mx, maxillary; na, nasal; oc, occipital condyle; ol, olecranon process (ulna); P and p, upper and lower premolars; pa, parietal; phi, intermediate phalanges; php, proximal phalanges; pl, palatine; pr, petrosal promontorium; pta?, patella?; pts, pterygoid shelf; px, premaxillary; ra, radius; sc, scapula; sp, extratarsal spur; sq, squamosal; st, sternal manubrium?; stl, distal styloid of tibia; sv, sacral vertebrae; tm, teres major process; tv, thoracic vertebrae; ul, ulna; and vsf, ventral scapular facet.

Meckelian groove of the mandible (Fig. 2, A and C). The cartilage is curved at mid-length, and its posterior limb is twisted relative to the anterior limb so the posterior end diverges away from the mandible (Fig. 2A, red and blue arrows). Its preserved part in *Maotherium* is more gracile than the Meckel's cartilage in situ with connection to the mandible in *Repenomamus* (*12*). It is identical to the Meckel's cartilage preserved in *Gobiconodon* (*13*) and the ossified Meckel's cartilage in *Yanoconodon*, which is connected to the ectotympanic and malleus (*15*). Thus, we infer that *Maotherium* has a similar, ossified connection between the mandible and the middle ear (Fig. 3C) as in eutriconodonts.

Basal mammaliaforms have both a postdentary trough and a Meckelian groove. The middle ear and its associated postdentary rod (partly homologous to the ossified Meckel's cartilage) are lodged in the postdentary trough. A classic problem of Mesozoic mammals is whether the absence of the postdentary trough would mean the loss of the connection between the middle ear and the mandible (*14*, *16*). In *Maotherium*, the middle ear is connected by Meckel's cartilage to the mandible without a postdentary trough. Thus, the absence of a postdentary trough cannot exclude the possibility that the middle ear was still attached to the mandible. It adds to the evidence that Meckel's cartilage is evolutionarily labile within the spalacotheroid clade. Because the Meckelian groove is present in the Early Cretaceous spalacotheroids *Maotherium* and *Zhangheotherium* but absent in the derived spalacotheroids of the Late Cretaceous (*6*), the adult retention of Meckel's cartilage occurred only in Early Cretaceous taxa of this clade and was likely lost in its Late Cretaceous members that show no trace of Meckel's groove (*6*).

Maotherium corroborates an earlier observation that its related genus *Zhangheotherium* has a disarticulated Meckel's cartilage (*12*), but the new fossil of *Maotherium* (HGM 41H-III-0321) shows that the Meckel's cartilage is connected to the mandible in spalacotheroids and adds new anatomical information. Compared with *Yanoconodon*, the Meckel's cartilage in *M. asiaticus* indicates that the middle ear is oriented at an angle to the mandible (Figs. 2C and 3). The mid-length curvature of Meckel's cartilage made it feasible for its anterior limb to be nestled in the Meckel's groove on the mandible, whereas its posterior limb was separated mediolaterally from the mandible (Fig. 2, A and C). From the curvature of Meckel's cartilage, it can be inferred that the ectotympanic ring and the malleus manubrium should be oriented obliquely to the vertical coronoid-angular part of the mandible (Fig. 2) (*15*). Observation of *Maotherium* and *Yanoconodon* suggests that, before its disconnection from the mandible, the ancestral middle ear was already mediolaterally separated from the mandible (*12*, *15*, *25*).

DMME in extant mammals is accomplished by two ontogenetic steps (*25*–*27*): first, a mediolateral separation of the middle ear anlagen from

the mandible in embryonic stages (seen in extant monotremes and placentals); second, a loss of connection to the mandible by reabsorption of Meckel's cartilage in fetal stages. In *Maotherium* and eutriconodonts, the mediolateral separation of the middle ear from the mandible had already occurred. However, Meckel's cartilage is ossified, and its reabsorption never happened, resulting in retention of a middle ear connection to the mandible otherwise seen only in the embryonic or fetal stage of extant mammals.

The middle ear of *Maotherium* and eutriconodonts shows a paedomorphic resemblance to the embryonic pattern of modern monotremes and placentals in which the middle ear is mediolaterally separated from the mandible but still connected via Meckel's cartilage to the mandible (*25*–*27*). All that is necessary for adult eutriconodonts and spalacotheroids to retain this paedomorphic mandibular ear connection is a relatively earlier timing in ossification of the Meckel's cartilage. The homoplastic separation of the middle ear from the mandible in Mesozoic mammals is correlated with ontogenetic heterochrony.

By parsimony of all characters (*1*–*6*, *15*), *Maotherium* and its spalacotheroid clade are more closely related to living therians than to multituberculates and eutriconodonts, all within Mammalia (Fig. 3) (*22*). *Maotherium* is similar to eutriconodonts in having the middle ear connected to the mandible in adults (Fig. 3 right side, DMME absent) but conspicuously different from living therians, multituberculates (*22*, *28*), and modern monotremes (and possibly *Hadrocodium*), in which the middle ear is separated from the mandible in adults (Fig. 3 left side, DMME present). The separation of the middle ear in mammalian phylogeny may have occurred by two alternative evolutionary scenarios: (i) DMME was present in the common ancestor of Mammalia (Fig. 3, node 3), but eutriconodonts and spalacotheroids reevolved the middle ear attachment to the mandible; or (ii) DMME was absent in the common ancestor of mono-

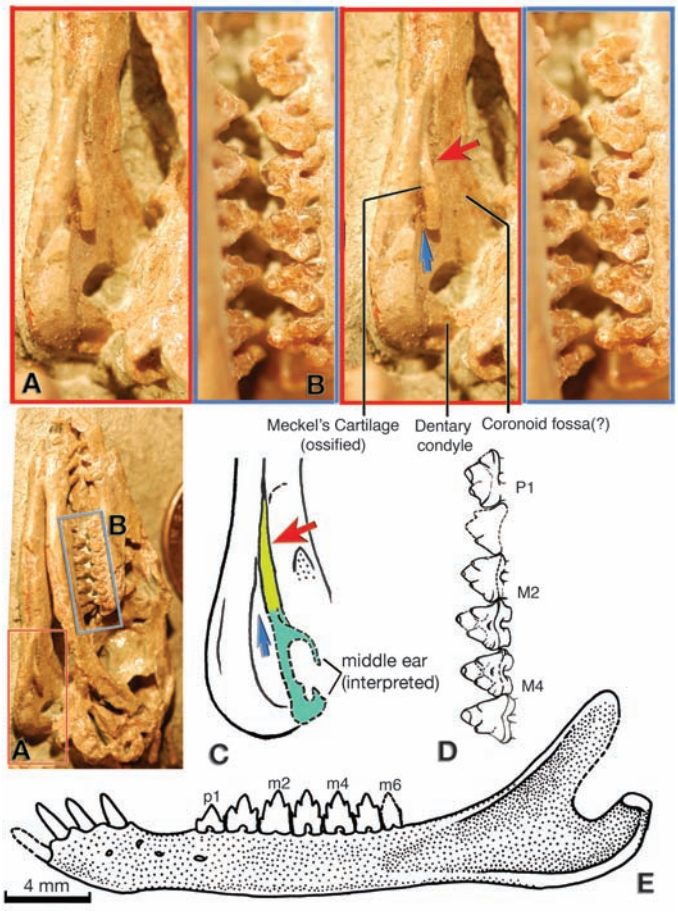

Fig. 2. Dentition and mandible of *Maotherium asiaticus* (HGM 41H-III-0321; holotype) and interpretation of its middle ear. (**A**) The preserved Meckel's cartilage (ossified) and its attachment to the mandible (stereophotos). (**B**) Upper postcanines (P1 to M5) (stereophotos). (**C**) Interpretive restoration of the middle ear (dashed line) and its attachment to the mandible [as in (A)]. Red arrow, mid-length curvature of Meckel's cartilage (identical to eutriconodonts); blue arrow, mediolateral separation; the ectotympanic and malleus are conjectural, based on the middle ears of multituberculates and *Yanoconodon*. (**D**) Upper premolar and molars [oblique view, as in (B)]. (**E**) Mandible (reconstruction, lateral view).

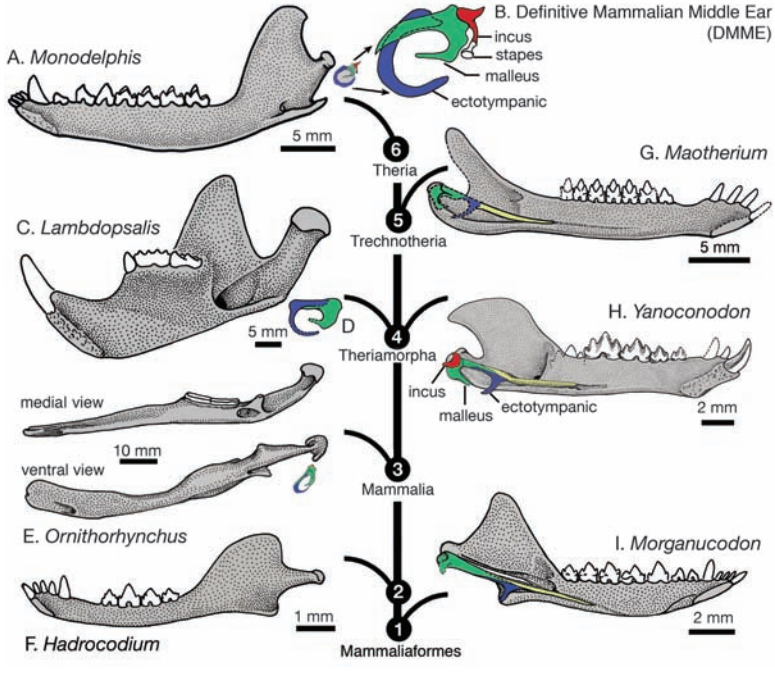

Fig. 3. Homoplastic evolution of the mammalian middle ear by paedomorphic retention of ossified Meckel's cartilage among extinct clades of mammaliaforms. Right, ancestral conditions of ossified Meckel's element in premammalian mammaliaforms, and in paedomorphic retention of ossified Meckel's cartilage in extinct clades of Mammalia. Left, living mammal and mammaliaform clades achieved the reabsorption of embryonic Meckel's cartilage and the DMME. (**A** and **B**) Extant therian *Monodelphis*: mandible [(A), medial view] and middle ear [(B) medial view]. (**C** and **D**) Extinct multituberculate mammal *Lambdopsalis*: absence of Meckel's cartilage on the mandible [(C) medial view] and separated middle ear ossicles [(D) ventral view] in adult [modified from (*28*)]. (**E**) Extant monotreme *Ornithorhynchus*: reabsorption of embryonic Meckel's cartilage and separation of middle ear from the mandible (top, medial view; bottom, ventral view) in adult. (**F**) Mammaliaform *Hadrocodium*: mandible (medial view) without Meckel's groove for middle ear attachment. (**G**) Partial restoration of the spalacotheroid *Maotherium asiaticus*: ossified Meckel's cartilage connecting the middle ear to mandible (medial view); hypothetical restoration of ear ossicle based on other Mesozoic mammals. (**H**) The eutriconodont *Yanoconodon* (and gobiconodontids): ossified Meckel's cartilage connecting middle ear to the mandible but ossicles partially (mediolaterally) separated from the mandible. (**I**) Mammaliaform *Morganucodon*: ossified Meckel's elements and the middle ear in the postdentary trough and Meckel's groove of the mandible. [Tree simplified from the phylogeny in (*22*)]; phylogenetic tree nodes 1 and 3 to 6 as labeled; node 2 (*Hadrocodium*) represents an unnamed mammaliaform clade.

tremes, eutriconodonts, and the living therians, and this ancestral feature is retained in eutriconodonts and spalacotheroids; but DMME evolved in extant monotremes (*14*) and in multituberculates for a second time, and then again for a third time in marsupials and placentals.

Paedomorphosis, or retention of fetal or juvenile characteristics of ancestors and/or relatives through developmental heterochrony, is a common phenomenon in vertebrate evolution. Scenario i gains support from the paedomorphic similarity of the ossified Meckel's cartilage in eutriconodonts and spalacotheroids to that of extant mammalian embryos (*15*). The premature ossification of Meckel's cartilage represents a simple developmental change in timing (heterochrony), for which a genetic mechanism has been established (*29*, *30*).

A mutant genetic and signaling network that can result in a premature ossification of Meckel's cartilage in mammalian embryogenesis has been characterized by recent genetic studies. Meckel's cartilage derives from cranial neural crest cells; it serves as scaffolding for development of mandibular and middle ear elements. Normal development of the Meckel's cartilage and its derivatives in vertebrates requires a wide range of structural and homeobox genes, such as *Bapx1*, *Gsc*, *Emx2*, *Sox9*, and *Type II Col*., which are expressed in the mammalian middle ear structure [reviewed by (*30*)]. Morphogenesis of Meckel's cartilage also requires a variety of growth factors, some of which are ubiquitous in mammalian development. This complex signaling network for Meckel's normal development includes transforming growth factors–β (*Tgf*-β) (*29*), connective tissue growth factors (*Ctgf*) (*29*), fibroblast growth factor (*Fgf*) (*30*), epidermal growth factor (*Egf*) (*31*), and bone morphogenetic proteins (*Bmp*), among others.

In normal chondrogenesis of Meckel's cartilage of *Mus* (wild type), the signaling pathway from *Tgf*-β (upstream) to *Ctgf* (downstream) stimulates the proliferation, and inhibits the terminal differentiation, of chondrocytes (*29*). Mutant *Tgfbr2* $^{fl/fl}$*;Wnt1-Cre* genes (a mutant of *Tgf*-β) accelerate chondrocyte proliferation and cause ossification of Meckel's cartilage in mutant *Mus* (*29*). The phenotype of ossified Meckel's cartilage in *Tgfbr2* $^{fl/fl}$*;Wnt1-Cre* mutant mice is similar to the fossilized and (prematurely) ossified Meckel's cartilage in the spalacotheroids (Fig. 2) and eutriconodonts (Fig. 3). This suggests that some similar developmental pathway had underlined the ossification of Meckel's cartilage in extinct Mesozoic mammals.

Among living mammals, an ossified Meckel's cartilage occurs only in certain mutant mice; the cartilage is retained only in pathological cases among humans. However, ossified Meckel's cartilage evolved at least twice in Mesozoic spalacotheroids and eutriconodonts. The absence of ossified Meckel's cartilage in the adult in extant monotremes, marsupials, and placentals represents a more-canalized development of the middle ear for these living lineages, in contrast to a much more labile evolutionary development of middle ear features, made possible by a greater diversity of about 20 Mesozoic mammaliaform clades (*1*, *5*).

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17. Systematics: Class Mammalia; Clade Trechnotheria (*1*); Superfamily Spalacotheroidea (*9*, *10*); Family *Zhangheotheriidae*; Genus *Maotherium* (*10*); sp. nov. *Maotherium asiaticus*. Holotype: Henan Geological Museum, Zhengzhou, China (HGM 41H-III-0321; Figs. 1 and 2), an almost complete skull, and most of the postcranial skeleton. Etymology: *asiaticus*, Latin for the Asiatic provenance of zhangheotheriids. Locality and Age: Lujiatun Locality (120°54′41″E, 41°36′26″N), Beipiao, Liaoning Province, China; the Yixian Formation. The site dated to be 123.2 ± 1.0 million years old (*22*). Diagnosis: I3, C1, P1, M5; i3, c1, p1, m6. *M. asiaticus* is referred to *Maotherium* by apomorphies shared by *Maotherium sinensis* (type species): deep precondylar notch of mandible, a deep ectoflexus on upper molars with cusp B′, a gradient in increasingly acute angle in triangulation of molar cusps toward the posterior molar, sigmoidal shape of the posterior ventral border of mandible. Differs from *M. sinensis* in having apomorphies of low central cusp between B′ and C; *M. asiaticus* has the tibial distal styloid process and the proximolateral process of the metatarsal 5 (all three features absent in *M. sinensis*). Full diagnosis in (*22*).
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Daily Electrical Silencing in the Mammalian Circadian Clock

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Neurons in the brain's suprachiasmatic nuclei (SCNs), which control the timing of daily rhythms, are thought to encode time of day by changing their firing frequency, with high rates during the day and lower rates at night. Some SCN neurons express a key clock gene, *period 1* (*per1*). We found that during the day, neurons containing *per1* sustain an electrically excited state and do not fire, whereas non-*per1* neurons show the previously reported daily variation in firing activity. Using a combined experimental and theoretical approach, we explain how ionic currents lead to the unusual electrophysiological behaviors of *per1* cells, which unlike other mammalian brain cells can survive and function at depolarized states.

In mammals, behavior and physiology are regulated on a daily basis by the brain's master circadian (~24-hour) clock in the suprachiasmatic nuclei (SCNs). The *period 1* (*per1*) gene is a key component of the molecular mechanism of this clock (*1*); its expression in the SCN peaks during the day and is low at night, and can be used as a marker of clock-containing SCN neurons and their circadian phase (*2*, *3*). SCN neurons are also thought to express time of day by changing their firing frequency, with high rates during the day and lower rates at night (*4*–*6*). A fundamental question in circadian biology is how the intracellular molecular circadian clock regulates the electrophysiology of SCN neurons. A Hodgkin-Huxley–type model of SCN neurons shows that circadian changes in ionic conductances can account for the circadian variation