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The gray mouse lemur (*Microcebus murinus*) as a model for early primate brain evolution

Chun Lum Andy Ho[1], Claudia Fichtel[2,3] and Daniel Huber[1]

Abstract

The gray mouse lemur (*Microcebus murinus*), one of the world's smallest primates, is thought to share a similar ecological niche and many anatomical traits with early euprimates. As a result, it has been considered a suitable model system for early primate physiology and behavior. Moreover, recent studies have demonstrated that mouse lemurs have comparable cognitive abilities and cortical functional organization as haplorhines. Finally, the small brain size of mouse lemurs provides us with actual lower limits for miniaturization of functional brain circuits within the primate clade. Considering its phylogenetic position and early primate-like traits, the mouse lemurs are a perfect model species to study the early evolution of primate brains.

Addresses

[1] University of Geneva, Department of Basic Neurosciences, Rue Michel Servet 1, 1206 Geneva, Switzerland

[2] Verhaltensökologie und Soziobiologie, Deutsches Primatenzentrum, Kellnerweg 4, 37077 Göttingen, Germany

[3] Leibniz Science Campus 'Primate Cognition', 37077 Göttingen, Germany

Corresponding author: Huber, Daniel (Daniel.Huber@unige.ch)

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Introduction

The primate brain has long been an object of fascination for neuroscientists. Not only because we as humans are part of the primate clade but also because of the remarkable encephalization that occurred during early primate evolution. The drivers behind this development, leading to exceptionally high brain-to-body ratios and parallel increased cognitive functions, remain a matter of intense debate and speculation. It is equally unclear what drove the initial development of other primate-typical features, including forward-facing orbits, fully opposable thumbs or limb specialized for reach grasping and leaping, and if these features are related to the evolution of brain size [1,2]. Here we summarize some of the current views and illustrate how the study of the world's smallest primate, the gray mouse lemur (*Microcebus murinus*), might provide us with precious insights into the early evolution of primate brains.

Arboreal lifestyle as a main driver behind primate traits

Primate evolution is invariably linked to trees. Roaming within the forest canopy and amongst a network of tree branches, our earliest primate ancestors ∼80MYA were probably arboreal. At that time, however, angiosperms mainly attracted insect pollinators to their flowers and produced mostly small seedlings that were only of little nutritional value. It would only be the early primate descendants who would develop a much more intimate and fruitful relationship with flowering plants. During the Paleocene–Eocene transition (∼56 MYA), which coincided with a global rise in temperature, angiosperms started producing a great variety of fruits and seeds including those adapted for animal dispersal [3]. Opportunistic about the newfound resources, our early ancestors most likely ventured out onto the terminal branches. These thin structures are however notoriously unstable and need to be held on to them while collecting fruits or pollinating insects [4], which probably drove the evolution of some of the primate-typical traits such as the ability to reach or grasp and opposable thumbs [5].

Today, the euprimate ancestors are long extinct, and their anatomy and behavior can only be reconstructed from fossil records [6]. These rare artifacts provide us with precious hints about their physiology and morphology and allow paleobiologists to build ever more sophisticated reconstructions of past ecosystems that inform us about the environment and challenges they faced.

A complementary approach is to study extant primates, which share similar environmental niches and physiology as extinct species, and hence have to cope with similar selective pressures. This approach allows to infer some plausible scenarios about the evolution of brain circuits, behavior, and cognition.

The mouse lemur as a model system for early primates

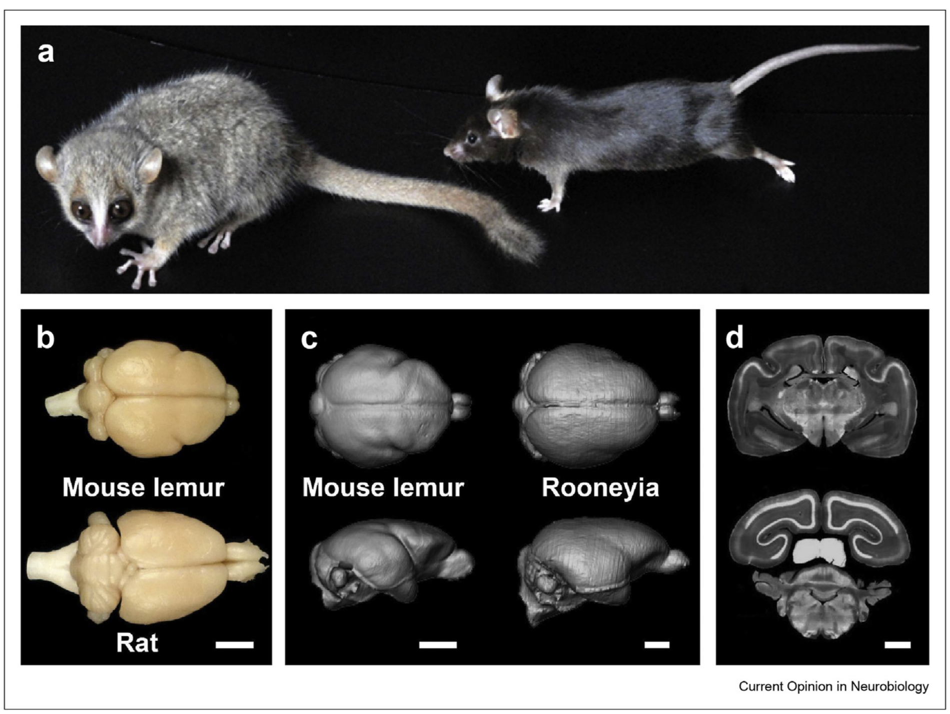
The gray mouse lemur (Figure 1A, left) is an interesting primate species fulfilling many of these criteria. Mouse lemurs are not only among the world's smallest primates ($\sim$60 g) and highly arboreal, they have also retained a number of primitive features such as long muzzles, wet noses, large olfactory bulbs, and lissencephalic brains (Figure 1B & C), features that almost certainly characterized the earliest primates [7]. Mouse lemurs belong to the strepsirrhines and are endemic to Madagascar. At present, 24 mouse lemur species are recognized [8]. Although today's lemurs are thought to represent the endpoints of an adaptive radiation following a single colonization event of Madagascar during the Eocene [9,10], the gray mouse lemur is particular because it has retained the size and generalist lifestyle of the original colonizers. It therefore provides a model of a basic framework of potential shared ancestral traits on which other primates built their specific-derived adaptations [11].

Since their first depiction in the late 18th century [12], mouse lemurs have been studied scientifically for over half a century [7,13]. This continued effort of generations of field biologists allowed detailed analyses of many aspects of their behavior and ecology [14–16]. In parallel, studies on captive mouse lemurs have revealed more details of their biology and physiology, including their metabolism [17,18], circadian rhythm [19], and genetics [20,21]. The possibility to study mouse lemurs under controlled laboratory environments was made possible owing to the initial and ongoing effort of the French National Museum of Natural History in Brunoy which established and maintained a research colony of mouse lemurs for over half a century [22]. Several other sister colonies have since been founded in France, Germany, and the United States of America, based on the animals from the Brunoy colony.

Anatomical and functional architecture of the mouse lemur brain

Having one of the smallest brains among extant primates, the mouse lemur brain morphology strikingly resembles that of ancient euprimates (Figure 1C). Based on three-dimensional endocasts reconstructed from skulls of an early ancestral euprimate, *Rooneyia*, a number of morphological similarities are evident, including overall size and shape, the aforementioned olfactory bulbs, as well as the presence of the Sylvian sulcus [23]. In contrast, the cytoarchitecture and internal anatomy of the euprimate brain remain obscure. Surprisingly, the anatomical structure of the mouse lemur's rat-sized brain (Figure 1B) carries most typical hallmarks found in larger

Figure 1



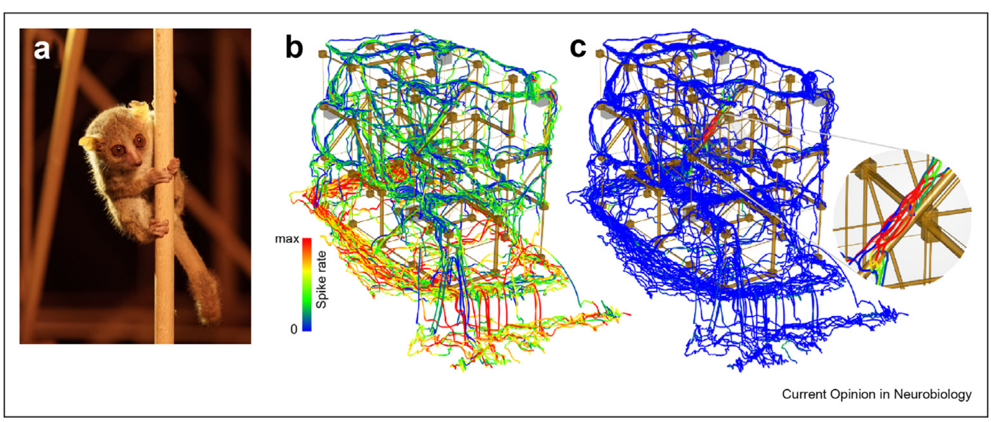
Body size and brain anatomy of the mouse lemur. (a) Size comparison between a mouse lemur (~70gr) and a mouse (~20gr). **(b)** A mouse lemur brain (top) compared with a rat brain (bottom). Scale bar: 5 mm. **(c)** Digital reconstructions of skull endocasts of a mouse lemur (*Microcebus rufus*) and of the early primate Rooneyia (adapted from the study reported by Kirk et al. [23], scale bars: 5 mm). **(d)** Coronal sections stained with the marker for vesicular markers of the vesicular glutamate show dense labeling in layer 4 of sensory cortices. Scale bar: 2 mm.

primates in both cortical and subcortical areas (Figure 1D). For example, the structure of the dorsal lateral geniculate nucleus of the visual thalamus revealed a clear six-layered structure [24,25] comparable to the one found in all larger primates studied so far [26]. Similarly, at the level of the primary visual cortex, typical patch-like cortical labeling [27], including cytochrome oxidase-positive blobs, have been described in the mouse lemur [28]. Given the early separation of the strepsirrhines around 60 MYA [29], it is thus likely that the early euprimate ancestor brain already carried many of the circuit elements still found in modern primate brains. In addition to classical histological techniques, the availability of a population-based magnetic resonance imaging atlas for the mouse lemur brain [30] or the application of cutting-edge tissue clearing histology will be important tools to compare brain structures across species.

Besides the basic anatomy, only little is known about the functional organization of the mouse lemur brain. The few studies carried out so far were specifically aimed at understanding the visual system. After the first description of the retinotopy of the mouse lemur visual cortex and basic connectivity with the thalamus using electrode recordings [24], a more recent study found striking parallels to other primate-typical visual features in the primary visual cortex [28]. Using intrinsic optical signal imaging, orientation preference maps with pinwheel-like arrangements were revealed. These structures shared similar statistical rules compared with all other primate species. Surprisingly, despite the small size of mouse lemurs, their orientation preference domains are similar in size even when compared with much larger primates including the macaque. This suggests an extremely weak scaling of basic computational units with body size within the primate clade. Finally, this study also demonstrated that methods, such as chronic optical imaging through cranial windows, initially developed for mice are easily transferable to the mouse lemur. This opens the door to explore population coding with single-cell resolution using, for example, functional imaging with two-photon microscopy.

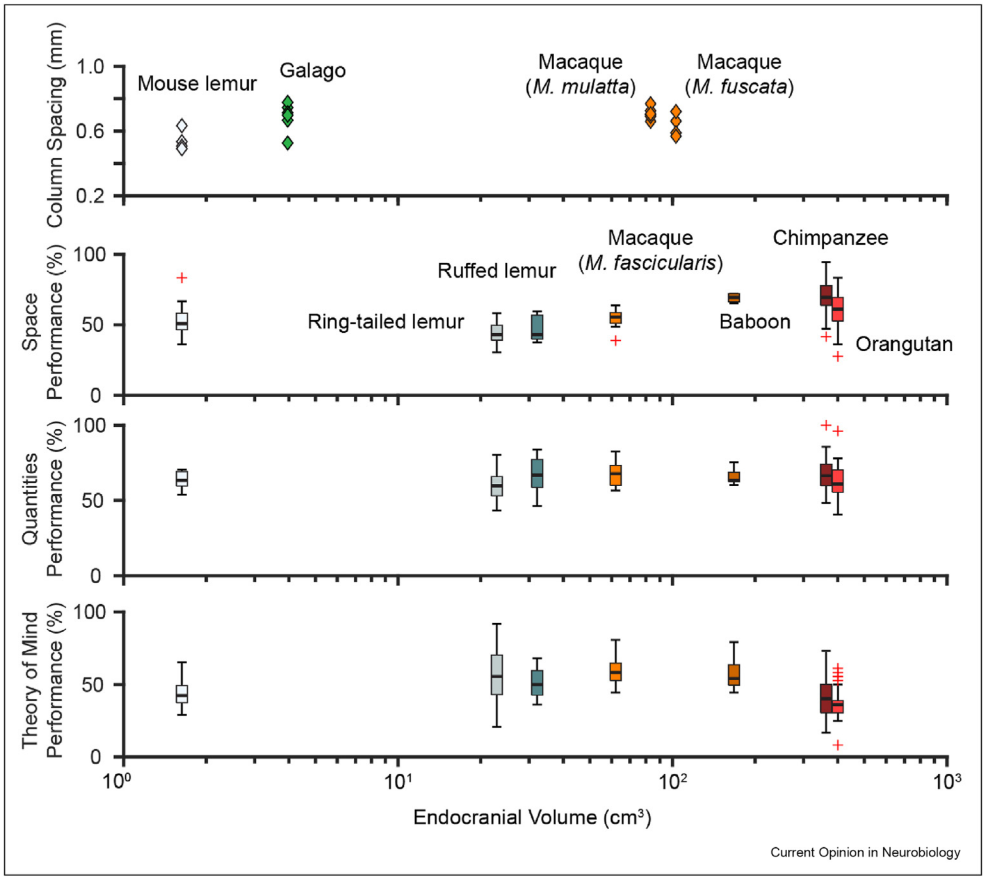
Whereas exploring their functional brain anatomy relies on relatively well-established research tools, studying their natural behavioral repertoire is a major challenge. In their natural habitat, decades of field observation have provided precious insights into their behavioral ecology [14–16], yet probing their behavior under more controlled settings is challenging. Trying to keep track of multiple animals chasing each other at high speed across the dense canopy in complete darkness, taking breathtaking jumps and disappearing regularly in tree holes makes this type of research a particularly difficult endeavor. In addition, their sensitive circadian cycle severely limits the use of any continuous artificial light outside their active period [31]. Recent development of infrared light video tracking methods has provided a possible solution (Figure 2, [32]. Using battery-powered and remote-controlled stimulus and reward dispensers positioned at multiple locations in the environment, this system ideally allows the study of mouse lemur ethology under controlled settings [32]. In combination with light-weight, wireless electrode recording devices such as behavior control systems allow to bring the laboratory into the wild or conversely establish a naturalistic setting in the laboratory (Figure 2).

Figure 2



Working with mouse lemurs in the laboratory. (a) A mouse lemur in an artificial lattice maze in the laboratory. **(b)** Wireless neuronal recording during a foraging session in the lattice maze synchronized with the animal's trajectory. The EthoLoop system is used to track the animal's location in three dimensions (3D) while providing behavioral triggered stimulus and reward using remote-controlled boxes (gray cubes, stick length 0.5 m). Example of one session where the 3D trajectory of an animal is color-coded based on the activity of two neurons recorded in dorsal CA1 hippocampus. Left; neuron showing increased activity when the animal is exploring the floor. **(c)** Another neuron recorded simultaneously with activity restricted to a single branch within the lattice maze (insert with higher magnification).

Figure 3



Comparison of brain size and cognitive performance between the mouse lemur and other primate species. (a) Visual cortex column spacing across primate species from mouse lemur to macaque is comparable and only scales weakly. **(b-d)** Cognitive performance of the mouse lemur in space (b), quantities (c), and theory of mind (d) scale in the primate cognition test battery is comparable to other primates despite almost three orders of magnitude difference in brain size (adapted from studies reported by Ho et al. [28] and Fichtel et al. [40], endocranial volumes from the study reported by Isler et al. [61], boxplot with median, quartiles, outliers in red + signs).

Small, but also smart?

Whereas probing of behaviors provides certain insights about their ethology, an important question still remains: too what extent can we compare the cognitive abilities of the rat-sized brain of the mouse lemur (or its equally sized ancestors) to those of larger primates?

Variation in brain size has traditionally been associated with cognitive abilities and behavioral flexibility [33]. However, it remains unclear whether brain size is indeed linked to performance in cognitive tests in primates. Addressing this type of question across species is inherently challenging. Testing procedures should be based on tasks adapted to the ethology of the subjects to capture variations in cognitive abilities rather than contextual variables or biases owing to motivational and attentional factors [34]. Touch screen-based testing procedures, for example, provide high-stimulus control and minimize operator—subject interactions, allowing testing in a wide range of primate species including mouse lemurs [35]. Nevertheless, they require extensive training with a high drop-out rate which most likely reflects motivational factors instead of variation in cognitive abilities.

An alternative to test the cognitive abilities of mouse lemurs is based on their leaping behavior [36]. Inspired by the Lashley jumping stand apparatus (initially developed for rats [37]), this task allows for robust single-trial learning with visual cues. Interestingly, access to their sleeping box was sufficient to act as a positive reinforcer. Performance by using this jumping stand procedure was better than in touch screen-based or go-nogo procedures [36]. In a similar vein, mouse lemurs' performance in spatial memory assessed by object permanence, rotation, or transposition tasks was inferior to chimpanzees or olive baboons, but research in the wild where mouse lemurs had to move between an array of feeding platforms revealed that they have an excellent spatial memory and exhibited a high travel efficiency in directed movements, most likely based on mental representations that are more detailed than a route-based network map [38]. These studies clearly highlight the notion of implementing appropriate experimental designs to evaluate cognitive abilities in animals to get a better understanding of how brain size is linked to performance in cognitive tests.

The primate cognition test battery is an established toolbox to compare cognitive abilities as it covers multiple physical and social domains [39]. Despite their small brains, mouse lemurs performed surprisingly on par when compared with chimpanzees and orangutans, which have a 200-fold larger brain (Figure 3, [40]). Whereas chimpanzees and orangutans performed better in cognitive tests on spatial understanding, mouse lemurs equaled them on causality or quantities. At the level of social cognition, mouse lemurs also performed and the apes even in tests of theory of mind [40]. Hence, these results question to some extent the notion of a clear-cut link between brain size and cognitive abilities. Indeed, a recent large-scale comparative study indicates that the largest brained mammals achieved relative brain size by highly divergent paths (i.e. differences in locomotor strategies) and not solely by selection on cognitive capacities [41]. Given that mouse lemurs' cognitive performance is on par with those of larger-brained primates, variation in the 'Bauplan' of brains might better explain variation in cognitive abilities.

Links between cognition and fitness

As a small solitary forager, facing one of the highest predation pressures among primates [42], the mouse lemur offers a unique opportunity to examine how cognitive abilities affect actual fitness and survival in the wild [43]. Being preyed on by nocturnal and diurnal species, including snakes, owls, and even related species such as the Coquerel's giant mouse lemur [42], avoiding predation is thus crucial. Mouse lemurs not only have an excellent spatial memory and travel efficiency [38,44] when navigating effectively between food resources and shelters (i.e. tree holes) but also exhibit highly flexible escape strategies adapted to the different hunting strategies of their predators [42]. Moreover, behavioral flexibility appears to be a key cognitive ability contributing to fitness. Flexibility, assessed via problem-solving abilities, is also correlated to one important fitness proxy predicting survival, that is, maintenance of body condition in a highly seasonal environment [43]. Hence, cognitive abilities, that is, behavioral flexibility, spatial memory, and travel efficiency are important capabilities directly associated with survival and fitness in mouse lemurs in the wild.

Small species age fast

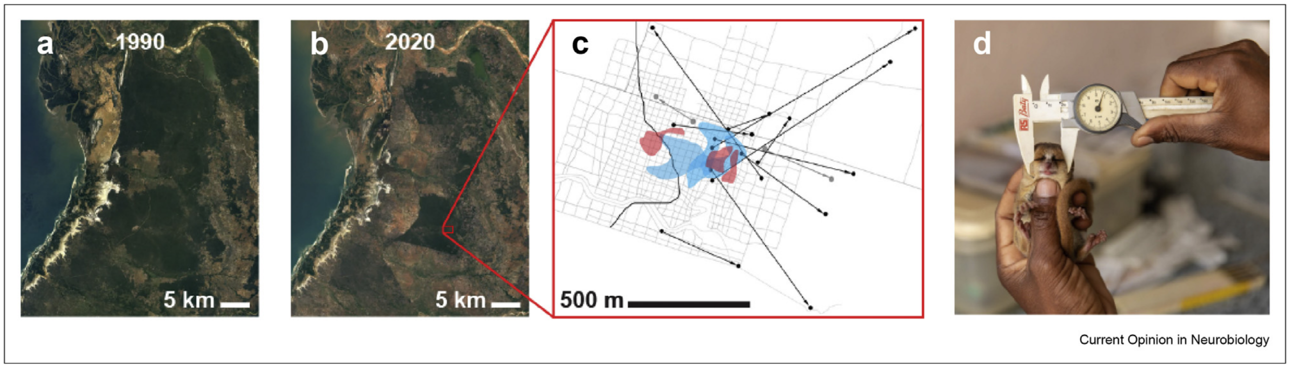
The small body size of the mouse lemur is also accompanied by a more rapid life cycle. Their development and aging are the fastest among primates [45]. Gestation only takes two months, and the pups are weaned after 6–8 weeks [22]. As mouse lemurs age, their sensory (e.g. developing cataracts [46]), motor, and cognitive abilities [47] begin to decline which in turn directly impacts survival. In the wild, the typical lifespan of a mouse lemur is only ~3 years [48]. Within the artificial settings of a managed colony, they can however reach up to ~14 years. By this point, age-associated changes in brain morphology, such as atrophy [49] and biochemical alterations, including tau protein-immunoreactive accumulation are present [50]. Interestingly, in around ~15% of aged mouse lemurs, neuropathological signs of Alzheimer's disease (senile plaques and neurofibrillary tangles) have been described [51]. Whereas these pathologies seem to be more prevalent in the unnaturally prolonged lifespan in captivity [52], it draws interesting parallels to the modern human condition where lifespan has substantially increased over the last two centuries [53], leading to a multitude of age-related global health issues. It is hence not surprising that the mouse lemur has been put forward as an ideal nonhuman primate model mimicking several aspects of human aging [54].

Outlook

Future studies involving the mouse lemur will also involve genetics. A recent initiative began to frame the mouse lemur as an upcoming model species for genetic studies for primate biology, behavior, and health [55]. The mouse lemur genome has recently been sequenced [20] and by developing a comprehensive phenotyping protocol including morphological, physiological, and behavioral traits, potential target genes can be identified for reverse genetic approaches [55]. As a model for aging and neurodegeneration, investigating the underpinning genetics with the mouse lemur will be an exciting avenue to pursue [56]. Because the gray mouse lemur is still among the most abundant primates in Madagascar (although, see the following), genetic data obtained from individuals might allow studying the genetic factors related to behavioral variation and fitness in the wild. Furthermore, at the population level, studying the mouse lemur and its closely related cousins can provide a glimpse on how adaptation alters the gene pool [21]. Owing to its phylogenetic position and its small size, the mouse lemur's genome may provide particularly precious information about primate brain evolution when compared with other species.

Finally, whereas the evolutionary aspects related to its phylogenetic position make the study of the mouse lemur brain particularly pertinent, there are also very practical advantages of working with mouse lemurs in the laboratory: breeding; holding; and handling are particularly easy compared with any other primate species, including the marmoset or macaques. Furthermore, most recording techniques and viral tools

Figure 4



Mouse lemur habitat degradation. (a and b) Aerial view of the Kirindy area in Madagascar in 1990 and 2020, respectively (adapted from Google Earth Engine [62]). Massive deforestation has reduced the western Madagascar dry forest corridor to small, isolated patches. Long-term field studies of the German Primate Center are concentrated on the area along the Kirindy River (small red insert). **(c)** Examples of home ranges (blue and red areas) and male mouse lemur dispersals (black dots and arrows) mapped by field studies illustrate the far distances which can be traveled by the small-sized animals (grey lines represent topographical landmarks such as rivers and footpaths, adapted from the study reported by Schliehe-Diecks et al. [15]). **(d)** Morphological measurements such as head-width that are typically taken during capture events in field studies (image credit A. Ozgul).

initially developed for rodents are easily transferable [24,28,32]. This provides a unique opportunity for the neuroscience community to address key questions in vision, navigation, sensory-motor coding, or decision making directly in a primate and thus substantially increase the transferability to humans. All these exciting prospects hinge however on the presence of a healthy and protected habitat.

Environmental urgency

Madagascar is one of the biodiversity hotspots with mainly endemic flora and fauna that suffer from an alarming rate of habitat destruction and fragmentation [57]. More than 90% of Madagascar's forests have disappeared, and the remaining forests, which served as important corridors, become increasingly fragmented owing to slash and burn agriculture (Figure 4A and B, [58]). The endemic lemurs of Madagascar are thus considered to be among the most endangered groups of mammals today [57,59]. A majority of the 24 mouse lemur species are considered endangered, and four species are critically endangered (The IUCN Red List of Threatened Species 2020). A handful of conservation programs protecting these species have been established, and a few long-term research projects provide important conservation benefits by increased monitoring and building support for the protection of their habitat [60]. Yet much more support from the scientific community and the public is required to assure the survival of the precious Malagasy biodiversity including mouse lemurs. In a philosophy similar to the conservation initiative of zoos (www.aza.org), we therefore urge all researchers working with or citing insights gained from work with mouse lemurs to actively engage in protecting their natural habitat before it is too late.

Conflicts of interest statement

Nothing declared.

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Using intrinsic signal imaging, the authors reveal orientation preference maps arranged into 'pinwheels' in V1 of *Microcebus murinus* that share similar map statistics as other primates. This study demonstrates that primate orientation column spacing only weakly scales to body size.

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In this study, the authors developed a real-time video tracking system to follow animals, including mouse lemurs, in naturalistic environments. Furthermore it allows interacting with the animals via remote controlled stimulus-reward boxes or optogenetic stimulation to shape reproducible behavior. In addition, the authors also demonstrate the integration with electrophysiology by recording place-like neural activity in the mouse lemur hippocampus.

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