

## **Supplementary methods: criteria to outline cerebral structures**

### ***Caudate nucleus***

In mouse lemurs, the caudate nucleus can be divided into the head, body, and tail. Different landmarks were used to outline these three parts. The head and the body of the caudate nucleus are bordered by the lateral ventricle medially, the internal capsule laterally, and the corpus callosum dorsally. A horizontal line drawn from the most ventral part of the lateral ventricle defined their ventral boundaries (Supplementary Fig. 1A). Rostrally, the head of the caudate nucleus was outlined until it was no longer visible.

The tail of the caudate forms an “arch” with its most caudal section corresponding to a region close to the body of the caudate and with the end of the tail being more rostral (Supplementary Fig. 1F). The caudal border of the tail of the caudate was outlined in the most caudal coronal slice where the hippocampus was divided into ventral and dorsal parts. At this level, the tail of the caudate was bordered laterally and dorsally by the corpus callosum. The fimbria and hippocampus were the medial and ventral borders (Supplementary Fig. 1B). The end of the tail of the caudate was defined by using an axial section that passed through the anterior commissure (Supplementary Fig. 1D). At this level, white matter separates the amygdala from the tail of the caudate. A straight horizontal line drawn through the center of the tail of the caudate served as a landmark on coronal sections to determine the last slice where the tail of the caudate was outlined (Supplementary Fig. 1D–E).

### ***Splenium of the corpus callosum***

The volume of the splenium of the corpus callosum was measured for evaluating the age-related decline in white matter and was estimated from five coronal slices. The caudal slice was the most caudal section where the corpus callosum passed through the sagittal plane (Supplementary Fig. 2A). Given that fibers of the cingulum bundle and dorsal hippocampal

commissure border on the splenium, the measure probably also included these two other structures.

### ***Septal region***

The cholinergic septal region was outlined from four coronal slices where the boundaries were easily identified. The lateral borders consisted of the two lateral ventricles, and the dorsal border was the corpus callosum. The anterior commissure served as the ventral limit (Supplementary Fig. 2B). The most caudal of the four slices was defined by the most caudal coronal section where the anterior commissure passed through the sagittal plane. The portion of the septal region measured contained the substantia innominata (where the nucleus basalis of Meynert is localized) and parts of the medial septal nuclei that provide the major cholinergic input to the cerebral cortex and the hippocampus (Mesulam, 2004). It also included parts of the dorsal and lateral septal nuclei.

### ***Hippocampus***

The hippocampus is of particular interest because of its core role in memory. In mouse lemurs, the hippocampus has the shape of a curved tube with a thin dorsal portion above the thalamus and a broader ventral portion lying in the temporal lobe (Supplementary Fig. 3F). The hippocampus can be divided into three regions (caudal, rostral ventral, rostral dorsal) for which different landmarks were used to outline the structure. The caudal part of the hippocampus was entirely surrounded by white matter (corpus callosum) and thus easily outlined (Supplementary Fig. 3A). The rostral dorsal hippocampus was bordered by white matter laterally, by the lateral ventricle dorsally, and by the third ventricle medially and ventrally (Supplementary Fig. 3B). The rostral ventral hippocampus was bordered by the internal (ITH) and external (ETH) part of the temporal horn of the lateral ventricle (Supplementary Fig. 3C). Its rostral border was determined by using the caudal end of the amygdala as a landmark. This boundary was easily determined on an axial section through the

superior limit of the optic chiasma by tracing a straight horizontal line passing on the caudal limit of the amygdala. This line served as a landmark on coronal sections. The most rostral slice where the ventral hippocampus was outlined was the one before the appearance of this landmark (Supplementary Fig. 3D–E).

### ***Cortical thickness***

The thickness of 12 different cortical areas (Supplementary Fig. 4) was estimated based on four coronal slices corresponding to +6.5 mm, +3.0 mm, + 0.5 mm, and -2,75 mm from the middle of the inter-ear distance (Bons et al., 1998). The 12 cortical regions that were measured (Table 1) could be grouped as the frontal, parietal, temporal, occipital, and cingulate cortices.

### ***References for supplementary methods***

Bons, N., Sihol, S., Barbier, V., Mestre-Frances, N., Albe-Fessard, D., 1998. A stereotaxic atlas of the grey lesser mouse lemur brain (*Microcebus murinus*), *Brain Res. Bull.* 46(1, 2), 1-173.

Mesulam, M.M., 2004. The cholinergic innervation of the human cerebral cortex, *Prog. Brain Res.* 145, 67-78.