Preliminary study on fine structures of subcortical nuclei in rhesus monkeys by ex vivo 9.4 T MRI

DEAR EDITOR,

Changes in fine structures of the brain over a life span can have robust effects on neural activity and brain function, which both play crucial roles in neurodegenerative diseases. Clinically, however, low-resolution MRI only provides limited information about fine brain structures. Here, using high-resolution 9.4 T MRI, we established a set of structural images and explored the fine structures of the claustrum, hippocampus, amygdala complex, and subregions of the amygdala complex (BLA, including lateral, basal, and accessory basal subnuclei) in rhesus macaque (Macaca mulatta) brains. Based on these high-resolution images, we were able to discriminate the subregional boundaries accurately and, at the same time, obtain the volume of each brain nucleus. Thus, advanced high-resolution 9.4 T MRI not only provides a new strategy for early diagnosis of neurodegenerative diseases, but also provides the ability to observe fine structural changes in the brain across a life span.

The basolateral complex of the amygdala (BLA), including the lateral, basal, and accessory basal subnuclei, is an important region receiving information from the cortex and subcortical nuclei, including the hippocampus, and transferring information to other parts of the amygdala (Huang et al., 2013; Pikkarainen et al., 1999; Sah et al., 2003; Saunders et al., 1988). Neuroimaging studies have significantly expanded our knowledge of brain structure and function during postnatal development and aging, as well as the progression of neurological and psychological disorders (Knickmeyer et al., 2010; Shaw et al., 2008; Sowell et al., 2002a, 2002b). The introduction of 9.4 T MRI technology has greatly facilitated subcortical classification of brain regions with fine dimensions (Kwan et al., 2017). With its high resolution, we can accurately discriminate subregional boundaries and obtain the volume of each brain nucleus. As an advanced imaging device, 9.4 T MRI provides a new strategy for the early diagnosis of neurodegenerative diseases and can further clarify fine structural changes in the central nervous system over a life span.

Three female rhesus monkeys aged from 12 to 20 years old were used in this study. Postmortem brains of the monkeys were provided by the Kunming Primate Research Center (KPRC) of the Chinese Academy of Sciences (CAS). Details on age and brain volume are provided in Table 1. The brains were acquired following approved protocols from the Kunming Institute of Zoology (KIZ), CAS. Briefly, the monkeys were deeply anesthetized with an overdose of sodium pentobarbital (50 mg/kg i.m.) and perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brains were removed from the skulls and submerged in a 500 mL solution of 4% paraformaldehyde (PFA). Each brain was positioned in an oval-shaped container filled with FOMBLIN® perfluoropolyether (Solvay Specialty Polymers, Italy). The container was generated by 3D printing and was adapted to the outer surface of the brain so that the brain tissue was held steady during scanning. Brains immersed in perfluoropolyether were vacuumed for at least 3 d under 0.1 atmospheric pressure to remove all air bubbles in the sample before the MRI scans. One half of each brain was selected and cut into 50 μm sections through the amygdala along the horizontal plane. Sections were stained with Cresyl violet. All animal care and experimental procedures were carried out in strict accordance with the guidelines for the National Care and Use of Animals approved by the National Animal Research Authority (China) and the Institutional Animal Care and Use Committee (IACUC) of KIZ, CAS, and were approved by the Ethics Committee of KIZ and KPRC, KIZ, CAS (AAALAC

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MRI was performed on a 9.4 Tesla 40 cm MRI system (a superconducting magnet of 9.4 T/400 PS, Agilent Inc., USA, equipped with Avance HD BioSpec console, Bruker BioSpin MRI GmbH, Germany) with a patented transceive coil. To acquire high-quality MRI images, a newly patented conformal radiofrequency (RF) coil (Chinese Patent application 201710191106.8) based on the standard quadrature RF excitation/receive configuration was used. To achieve high sensitivity, the coil was designed with half open brain-conformal surface mode and composed of 16 distributed resonant elements with quadrature ports. Therefore, only one $^1$H channel was used but with sufficient sensitivity for high-resolution imaging. The maximal inner diameter of the coil was 95 mm, which is appropriate to hold the container and produce RF-illuminating volume for the entire brain.

For each macaque brain, $T_2^*$ weighted images of FLASH (Fast Low Angle Shot) 2D and 3D sequences were acquired. The 2D images were acquired with the following settings: TR/TE=2 900 ms/14 ms, flip angle=80°, resolution=208 $\mu$m×182 $\mu$m, slice thickness=0.5 mm, matrix size=384×384, 104 slices, acquisition time=18 m 30 s, band kHz. The FLASH 2D images were acquired eight times separately and averaged to achieve high-resolution images with a better signal-to-noise ratio (SNR). For the 3D images, the following settings were applied: TR/TE=45 ms/13 ms, flip angle=10°, voxel size=155 $\mu$m×155 $\mu$m×155 $\mu$m, matrix size=515×420×354, acquisition time=1 h 51 m 30 s, band kHz.

Currently, there is no precedent for subfield segmentation of the amygdala. In addition, standard analysis software cannot perform automatic segmentation of the amygdala subfields at the above resolution. Manual segmentation of 9.4 T images can be achieved with reference to an atlas (Saleem & Logothetis, 2012) using the ITK-SNAP package. Here, MRI segmentation of the hippocampus, amygdala, basolateral complex in the amygdala (BLA, including the lateral, basal, and accessory basal nuclei), and claustrum were manually performed with reference to the atlas “A Combined MRI and Histology Atlas of the Rhesus Monkey Brain in Stereotaxic Coordinates” (Saleem & Logothetis, 2012). The atlas, which also contains histological sections and images, clearly shows the boundaries of each nucleus for segmentation. The segmented regions were only regions of interest (ROI) and did not exactly match specific histological nuclei. The subregions of interest in the amygdala (sROI) were close to the BLA (ROIs of lateral nucleus, basal nucleus, and accessory basal nucleus, briefly denoted as L, B, AB, and claustrum).

Table 1 Specimen information

| Specimen ID | Age (Years) | Brain volume (mL) | Cerebellum volume (cm³) | Hippocampus | Amygdala | Lateral nucleus (L) | Basal nucleus (B) | Accessory basal nucleus (AB) | Claustrum |
|-------------|------------|------------------|------------------------|------------|----------|--------------------|-----------------|-----------------------|----------|
| 05076       | 12         | 73               | 8.02                   | 482.868    | 477.4    | 192.81             | 196.56          | 54.34                 | 61.74    | 36.71                | 42.48    | 59.2                  | 60.68    | 170.345               | 176.97   |
| 00006       | 18         | 93               | 8.57                   | 528.901    | 539.1    | 193.62             | 204.06          | 50.46                 | 58.59    | 46.22                | 44.61    | 61.72                 | 60.67    | 200.424               | 182.19   |
| 98374       | 20.3       | 93               | 8.22                   | 528.901    | 560.6    | 253.79             | 262.58          | 63.51                 | 70.98    | 38.82                | 38.45    | 66.24                 | 67.37    | 172.501               | 186.77   |
observed the fine structures in the amygdala, hippocampus, and BLA in the rhesus macaque brain. In contrast, the fine structures were not sufficiently clear for segmentation when using 3 T MRI. Clinically, psychological disturbances related to emotion and memory usually involve the amygdala, hippocampus, and their association (Sharp 2017; Yang & Wang, 2017). The BLA is the information entrance to the amygdala from the cortex and subcortical regions, such as the hippocampus. The lateral nucleus in the BLA is responsible for receiving extrinsic sensory information from the sensory thalamus and sensory cortices, and for output to other amygdala nuclei, including the basal and accessory basal nuclei (Janak & Tye, 2015; Pitkanen & Amaral, 1998). The BLA has reciprocal connections with the cortex and...
hippocampus, and thus information from the BLA can have a broad influence throughout the cortex (Saunders et al., 1988). Thus, by segmentation of all deep nuclei within fine brain structures, we can accurately obtain their volumes.

The current study was based on high-resolution 9.4 T MRI scans of ex-vivo rhesus macaque brains. The next step will be to undertake anatomical research of rhesus macaque brains in-vivo and to obtain high-resolution MRI images over their entire lifespan. Such research can supplement histological studies, which can lose information on fine structural changes over long periods of time. In addition, because of the large volume of brain tissue in rhesus monkeys, tissue removal by optical techniques is not ideal for understanding the spatial structures of deep brain nuclei. Therefore, high-resolution MRI images are essential for studying the fine structures of the brain, especially deep nuclei.

In conclusion, our findings suggest that the use of ultra-high-resolution MRI images to display the microstructures of the brain is an essential technique. In the future, it would be interesting to identify the connections among subcortical nuclei in the brains of live macaques following behavioral training and testing using simultaneous fMRI.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

J.L.L. and K.Z. initiated the project. H.Z.C. and H.Y.Y. designed the experiments. J.L.L. organized and supervised the whole project. H.Z.C. collected macaque brain samples and performed perfusion and fixation; H.Y.Y. and H.Z.C. performed MRI scanning and data collection; H.Z.C., K.Z., and J.L.L. drafted the manuscript, with input from all other authors. All authors read and approved the final version of the manuscript.

Huan-Zhi Chen¹,³, Hong-Yi Yang², Kai Zhong²,⁵, Jia-Li Li¹,⁴,⁶,*

¹ Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
² High Magnetic Field Laboratory, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, Anhui 230031, China
³ Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan 650223, China
⁴ CAS Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, Yunnan 650223, China
⁵ CAS Center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences, Hefei, Anhui 230031, China
⁶ Kunming Primate Research Center of the Chinese Academy of Sciences, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, China

*Corresponding author. E-mail: lijiali@mail.kiz.ac.cn

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