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A Cross-Species Brain Magnetic Resonance Imaging and Histology Database of Vertebrates

Tomokazu Tsurugizawa [1,2,3,6], Yuji Komaki [4,6], Illia Aota [5], Makoto Suematsu [4], Chiaki Ohtaka-Maruyama [5] & Takuma Kumamoto [5] ✉

Understanding brain evolution requires detailed comparative analyses of brain structures across species. However, high-resolution anatomical and connective data remain limited for most vertebrates beyond a few well-studied model organisms. To address this gap, we collected postmortem brain samples from a range of vertebrates, primarily small amniotes, and performed magnetic resonance imaging and histological staining. Here, we present the "Animal Brain Collection (ABC)," a freely accessible database that enables researchers to examine and compare cellular and tissue-level brain architectures across species. This resource provides a foundation for cross-species investigations of brain structure and development, offering new opportunities for research into the diversity and evolution of vertebrate brains.

Background & Summary

Determining the mechanisms of brain evolution by studying only a select number of organisms that have adapted to a restrictive survival environment is currently an inadequate approach. Here, we attempted a comparative analysis of the brain structures across several closely related species, highlighting a critical collection of data for researchers to understand seemingly trivial evolutionary developmental processes. With the advent of improved lineage tracing methods, cell sequencing technology, and whole-brain imaging techniques, a new molecular characterization of evolutionary processes has emerged. Current technologies, including single-cell transcriptomics, could help us identify equivalent cell types in mammalian and non-mammalian vertebrates, and studies on the anatomical brain structure and connectome could provide answers on how different species process complex cognitive and sensorimotor challenges. Among the techniques that have been utilized within the recent decade, magnetic resonance imaging (MRI) has greatly facilitated the visualization of the central nervous system in various animal species[1]. In general, T1/T2 structural imaging provides detailed images of vertebrate mammalian brains with different tissue structures. Recently, several studies demonstrated the myelin-rich areas of the brain using T1-weighted images, which facilitated the identification of specific parts of the visual cortex and tracking of changes in the myeloarchitecture across the cortex[2,3]. Contrarily, T2-weighted images emphasize the protons in the water fractions of the cerebrospinal fluid and gray matter. T2-weighted image is valuable for detecting various brain pathologies, including ventriculitis, multiple sclerosis, and brain tumors, making it an essential tool in neuroimaging[4]. Diffusion-weighted imaging measures the diffusion of water molecules throughout a tissue, allowing researchers to identify the orientation of white matter fiber tracts in the brain[5,6]. Although, compared with traditional microscopy, MRI might lack fine resolution required for deriving substantial conclusions regarding evolutionary changes of brain structures. However, MRI holds several advantages over conventional histology techniques. For example, MRI does not require tissue sectioning and labor-intensive tissue processing necessary for histology, and the scanned images do not require aberrant calculated corrections from tissue shrinkage. Furthermore, as the scanned images are organized digitally, the brain structures and neural fiber tracts can be viewed from multiple axial planes, and the measurements can be

[1]National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan. [2]Institute of Systems and Information Engineering, University of Tsukuba, Tsukuba, Ibaraki, Japan. [3]Université du Québec à Trois-Rivières, Trois-Rivières, Canada. [4]Central Institute for Experimental Medicine and Life Science, Kawasaki, Kanagawa, Japan. [5]Developmental Neuroscience Project, Department of Basic Medical Sciences, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan. [6]These authors contributed equally: Tomokazu Tsurugizawa, Yuji Komaki. ✉e-mail: kumamoto-tk@igakuken.or.jp

done by the computer, facilitating more nuanced interspecies comparisons. Thus, MRI is useful for investigating not only the pathophysiology of a human disease but also the brain and neural structure and function of other species, such as monkeys, rodents, rabbits, and reptiles[7], essentially performing a comparative analysis of their evolutionary anatomy in a live imaging setting. Although some studies have explored brain structure and function across species using MRI[8–11], additional research is needed to elucidate the diversity and conserved features of brain organization.

Careful curation of brain atlases facilitates comparative evolutionary anatomical studies as they aid in the visualization of species-specific brain structures. Thus far, several textbooks, such as the "The central nervous system of vertebrate" and "Principles of Brain Evolution" have greatly influenced the field of comparative neurobiology[12,13]. To date, curated resources available to these researchers now often take on the form of online databases. For example, BrainMaps.org contains high-resolution brain and neural images of a variety of mammalian, avian, and fish species, (http://brainmaps.org/index.php), and the Comparative Mammalian Brain Collection is one of the world's largest data collections of mammals (http://brainmuseum.org/index.html). Another online resource called the BSI-Neuroinformatics includes nine different databases (Degu-3D atlas, Marmoset-3D atlas, Marmoset-the MRI standard brain, Japanese Macaque Monkey-the MRI standard brain, BrainTx, Neurotycho, CelLoc-3D, Mathematical Neuroscience, and The autism CNV database) hosted by RIKEN Center for Brain Science (https://bsi-ni.brain.riken.jp/dbtools). The Digital Brain Bank, an open-access platform for postmortem imaging datasets on mammals, was introduced in 2021[9]. It provides MRI and tissue staining data for as many as 45 mammalian species at the time of publishing. This database is valuable for facilitating interspecies comparisons of the mammalian brain. These are just few of the many well curated databases available for comparative evolutionary researchers. In comparison to the relatively large brains of mammals, the smaller size of the non-mammalian brains poses challenges for sampling and impedes the acquisition of high-resolution MRI. The few existing atlases on non-mammalian vertebrates are rare and often limited to a low-resolution scale. For example, Brain-development.org (https://brain-development.org/) is a database of neonatal human brain images acquired by MRI. Scalable-brain-atlas (https://scalablebrainatlas.incf.org/index.php) provides unified access to many brain images, including those of macaques, mice, rats, humans, ferrets, marmosets, and opossums, as well as templates for different species through a set of web services.

Although imaging of brain morphology and neural connections are important, gene expression patterns are also important for elucidating the brain evolution patterns. There are some databases indicating certain gene expression patterns by *in situ* hybridization within the cortex. Allen Brain Atlas (mouse and human; https://portal.brain-map.org/), Genepaint (mouse; https://gp3.mpg.de/), MGI (mouse; https://www.informatics.jax.org/), Geisha (Chick; http://geisha.arizona.edu/geisha/), Xenbase (Xenopus; https://www.xenbase.org/entry/), and ZFIN (zebrafish; https://zfin.org/) show common neural transcription factor staining in fixed images. Recently, the MRI datasets pertaining to animals and humans have been made available worldwide. Data sharing will accelerate the studies because the researchers can easily reach the target images from their laboratory. The MRI databases of humans and rodents are available (https://www.humanconnectome.org/, https://www.humanconnectome.org/). The Allen Brain Atlas (https://portal.brain-map.org/) is useful for the fusion of *ex vivo* histology and MRI. However, a limited database contains a collection of various mammalian and non-mammalian brain images. Brain Catalogue (https://braincatalogue.org/) is a useful database, which is developed by analyzing the MRI data of large mammalian brains. It contains the data of 37 unique animal brains scans. While these useful databases have widely expanded in recent decades, a database for amniote brains, especially those of birds, reptiles, and small mammals, is not well established. In the present study, we established a novel MRI and histological database, termed *Animal Brain Collection* (*ABC*), which primarily comprises data from amniote species—including small mammals, birds, and reptiles—but also incorporates several representative non-amniote species (Figs. 1 and 2, Tables S1 and S2). The source database was created by ToGoDB (http://togodb.org/), an open-source database provided by the Database Center for Life Science (https://dbcls.rois.ac.jp/index.html). Although the exact time of death is unknown for some individuals—especially those discovered after an unspecified postmortem interval—the developmental stage is known and accurately annotated in the database. For non-adult specimens, the postnatal age in weeks is also provided in the database when available. At the time of submission, we have obtained the data for 44 collections across 29 species, and this database is continually being updated as new samples are obtained.

MRI, which enables non-invasive measurements, and histological staining or immunostaining, which facilitates confirmation of cellular structures and gene expressions, are highly compatible techniques. The combination of the sequential analyses from MRI and histological staining of the same brain samples allows for the confirmation of both complex brain structures as well as neural connections, cell structures, and gene expressions, contingent on the condition of the sample. The ABC database provides researchers with convenient and free access to raw data, allowing them autonomy in utilizing any data that they need. Recently, scRNA-seq and spatial transcriptome analyses of the non-mammalian brains, such as those of axolotl[14,15], salamander[16], turtle[17], bearded dragon[18,19], chick[20–22], and songbird[23], were reported. Beyond gene expression and anatomical comparisons between species, the combination of these genome-wide analyses and the ABC database will allow linking of the connectomes between the intra- and extra-cerebral regions and construction of neural circuits, providing new insights into the evolution of the vertebrate brain. We will continue to augment the number of analyses and sampling on smaller amniotes. Owing to the constraints of MRI, the brain samples of insufficient size will only have histological data. Nevertheless, we believe that they will still be a valuable resource in elucidating comparative brain evolution. Most MRI brain databases focus on rodents and non-human primates, as these species are commonly used in biomedical research. However, understanding brain evolution requires data from a wider range of animals. MRI data from other species remain limited, partly because their collection often involves collaboration across zoological institutions,
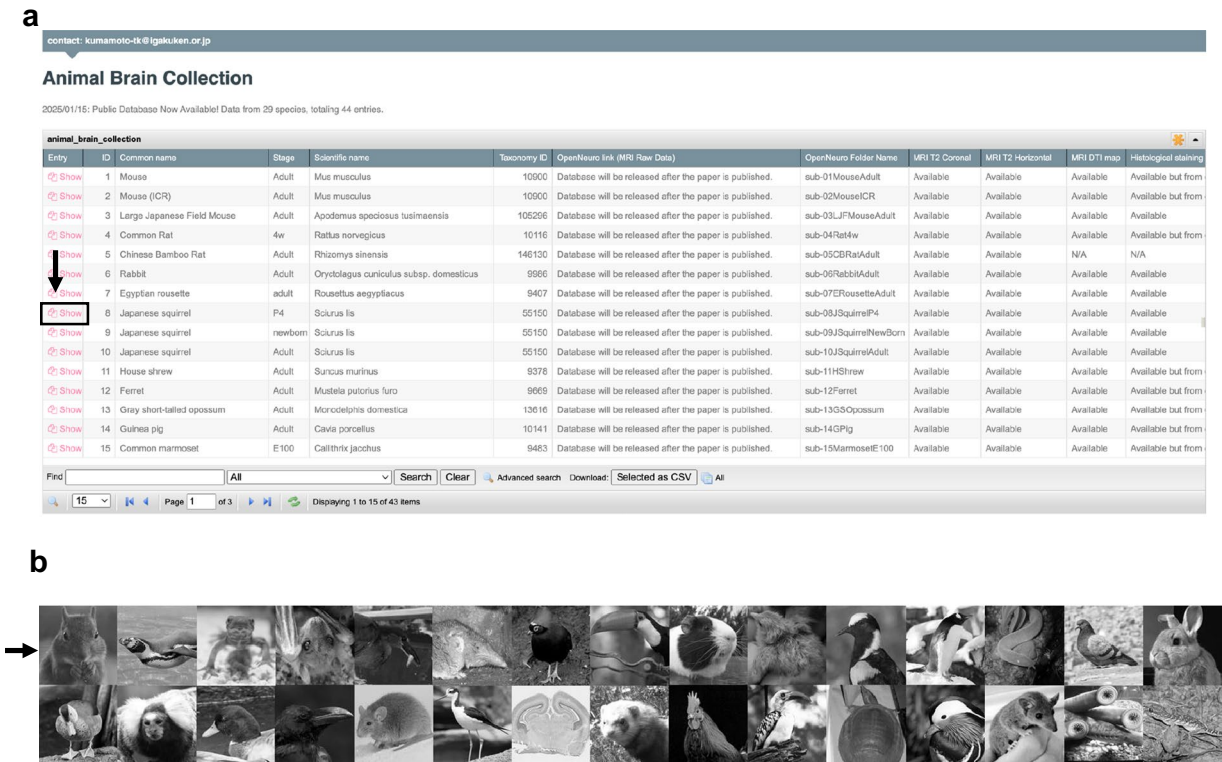
Fig. 1 Overview of the Animal Brain Collection (ABC) database. (**a**) Screenshot of the top page of the ABC database website, showing the user interface and navigation menu. (**b**) Representative images of animal species included in the database. The ABC primarily comprises data from amniote species—including small mammals, birds, and reptiles—and includes several non-amniote species. The detailed species list is provided in Table S1.

imaging facilities, and comparative neuroscientists. In the current study, we collected most of the brains from animal zoo and got the images using 7 T and 3 T MRI systems. Diffusion MRI and T1/T2-weighted images in postmortem animals give information of 3-dimension structure. Additionally, this update incorporates smaller cerebral samples, which are typically challenging to analyze using MRI, into the database. In particular, Diffusion Tensor Imaging (DTI) reveals the white matter architecture composed of myelinated bundles across the whole brain, including major commissural structures, such as the corpus callosum, and the anterior, posterior, and hippocampal commissures. The development of commissural and association fibers plays a crucial role in shaping brain connectivity, with these patterns of fiber organization deeply linked to the overall structural evolution of the brain. In this context, interspecies comparisons of fiber structures derived from MRI data provide a valuable means of investigating the formation of neural circuitry. Moreover, by employing ABC, it becomes possible to infer the brain architectures of an unprecedented range of species. This approach holds significant promise for advancing our understanding of brain structure not only in mammals and birds but also in reptiles and other vertebrates, thereby contributing to broader research on brain evolution.

Methods

Brain samples. Fixed brain samples were kindly obtained from Nasu Animal Kingdom and Kobe Animal Kingdom. The samples from other animals, such as ferret, opossum, skunk, and Madagascar day gecko, were obtained from RIKEN Center for Biosystems Dynamics Research (BDR) and kindly gifted by Drs. Fumio Matsuzaki and Hiroshi Kiyonari. The large-billed crow was kindly gifted by Dr. Masato Aoyama at Utsunomiya University. The samples of lamprey and hagfish were kindly gifted from Dr. Fumiaki Sugahara at Hyogo Medical University. The samples of bearded dragon were kindly gifted from Dr. Pei-shan Hou at National Yang Ming Chiao Tung University. Chinese softshell turtles were purchased from Yamato Yoshoku, chicken eggs from Yamagishi, and mice from SLC. All brain specimens, except for mice, chick, and turtle, used in this study were obtained postmortem, with no procedures performed on live animals. Therefore, ethical approval was not required as per the guidelines of our institution. The fixed brain samples were kindly provided by the following institutions: Nasu Animal Kingdom, Kobe Animal Kingdom, RIKEN BDR, Hyogo Medical University, and Utsunomiya University. Material Transfer Agreements (MTAs) between these institutions and ours are currently being processed to formalize the transfer and use of the materials and are expected to be finalized before publication.
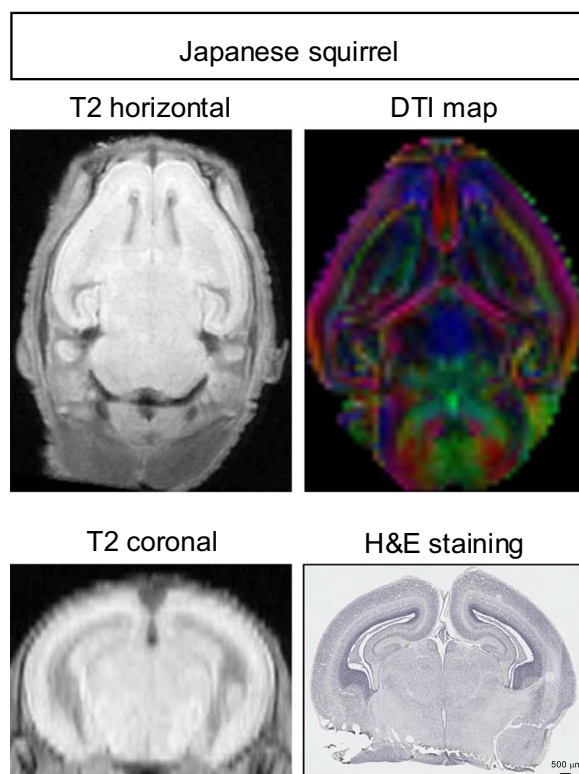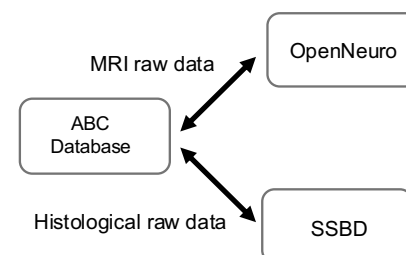
Fig. 2 Example of species-level data access in the Animal Brain Collection (ABC) database. (**a**) Interface displayed upon selecting the Japanese squirrel from the species list in Fig. 1a,b. Users can browse species-specific information, including sample metadata and data availability. (**b**) Example images available for each species, including T2-weighted MRI and corresponding histological sections. (**c**) Information regarding the acquisition of high-resolution raw imaging data is provided. All raw datasets have been deposited in OpenNeuro and the SSBC (RIKEN BDR Brain Atlas), with corresponding accession numbers available on the ABC database website.

MRI acquisition protocol for *ex vivo* fixed brain samples. MRI measurements of *ex vivo* fixed brains were performed following a previously reported protocol[24], with adaptations made according to the scanner type and sample size.
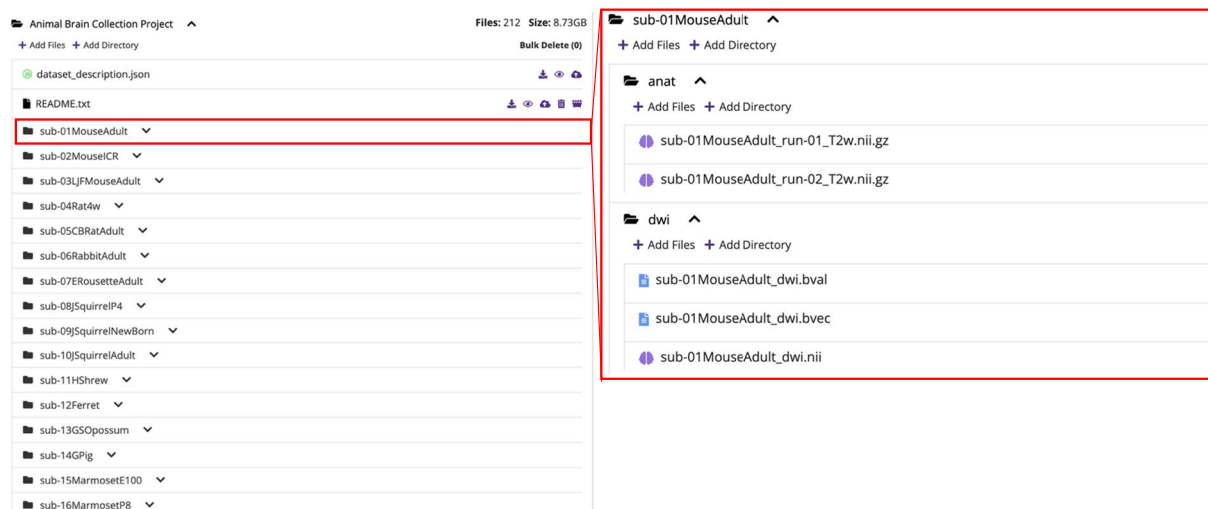
Framework of OpenNeuro

Fig. 3 Data sharing framework via OpenNeuro. Schematic representation of the data submission and access pipeline for the Animal Brain Collection (ABC) dataset via OpenNeuro.

1. ***Sample Preparation***

- Brain tissues were fixed in 4% paraformaldehyde (PFA).
- Before MRI scanning, samples were immersed in a 0.2 mM gadolinium-containing 0.1 M phosphate-buffered saline (PBS PH: 7.4) for at least one week.
- During MRI measurements, samples were immersed in Fluorinert (Sumitomo 3 M Limited, Tokyo, Japan) to minimize background signal.

2. ***MRI Scanners and Coil Selection***

a. For Small to Medium-sized Brains

- Scanner: 7 T MRI scanner (BioSpec 70/16, Bruker Biospin GmbH, Ettlingen, Germany)
- Gradient System: Actively shielded gradients with a maximum strength of 700 mT/m
- Coils:

    O Volume coils: φ 22 mm, 38 mm, 62 mm, and 72 mm (Bruker Biospin GmbH, Ettlingen, Germany)
    O Solenoid coils: φ 16 mm and 30 mm (Takashima Seisakusho, Tokyo, Japan)
    Coil selection was based on sample size.

b. For Large Brains (e.g., bamboo mouse)

- Scanner: 3 T Philips MRI scanner (Ingenia, Philips, Netherlands)
- Coil: 32-channel head coil

3. ***Imaging Sequences and Parameters***

a. 3D Anatomical Imaging (rapid acquisition with relaxation enhancement (RARE) sequence)

- Method: RARE
- Parameters:

    O Echo time (TE): 20 ms
    O Repetition time (TR): 300 ms
    O RARE factor: 4
    O Number of averages: 1–8 (adjusted to maintain signal-to-noise ratio > 20)
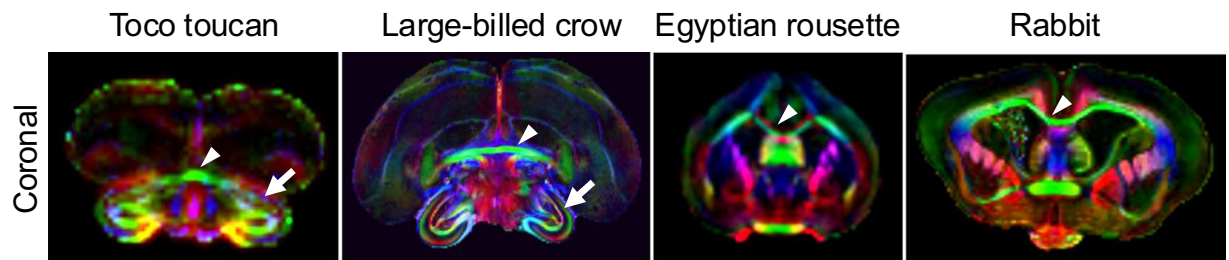   O Spatial resolution: 100–200 μm (depending on sample and scanner)

Fig. 4 Comparative brain anatomy in selected avian and mammalian species. Coronal T2-weighted high-resolution MR images of the brains from the large-billed crow, toco toucan, Egyptian rousette (fruit bat), and rabbit highlight interspecies differences in commissural fiber organization. Notably, the anterior commissure is prominent in the avian brains, whereas the corpus callosum is well-developed in mammalian brains (indicated by arrowheads).

b. 3D DTI

- Method: Spin echo-based DTI
- Parameters:

O TE: Minimum
O TR: 200 ms
O MPG gradient duration (δ): 3.5 ms
O MPG gradient separation (Δ): 9.5 ms
O b-value: 2000 s/mm²
O Diffusion directions: 30
O Number of 1 b0 images: 5
O Number of averages: 1
O Spatial resolution: 100–200 μm

MRI data analysis.   The position, orientation, and angle of the T2-weighted images were modified using SPM12 (Wellcome Trust Center for Neuroimaging, UK). Each brain section was extracted, and the fractional anisotropy (FA) was calculated from diffusion-weighted images using DSI Studio (http://dsi-studio.labsolver.org).

Histological staining.   For the H&E staining, isopropanol was added to brain slices, which were incubated for 1 min. After air drying the slide, hematoxylin (Sigma-Aldrich) was added and the samples were incubated for 7 min. The slides were washed with ultrapure water three times and a bluing buffer was added (DAKO). After incubating for 2 min, the slides were washed and incubated for 1 min with Eosin (Sigma-Aldrich). The slide was then washed once and mounted with Entellan New (Sigma-Aldrich) after dehydration. For the DAPI staining, after air drying the slides, the slides were washed with PBS to remove the Optimal Cutting Temperature (O.C.T.) compound, and, then, the samples were incubated with DAPI (1:2000 in PBS) for 15 min at room temperature The slides were sequentially washed in PBS three times, and then mounted with Mowiol (Sigma-Aldrich). The images were acquired by BZ-800 (Keyence). Images were exported in Tagged Information File Format (TIFF).

Data Records
The datasets are available at OpenNeuro[25] and SSBD[26].

MRI data deposit.   MRI analysis was performed to generate coronal and horizontal images, including T1/T2-weighted scans and DTI movies. Raw data are uploaded to OpenNeuro[25], where researchers can access the raw data so as to create a high-resolution image (Fig. 3 Table S3).

The following 44 MRI data files, derived from various species, have been deposited in OpenNeuro. The file structure is shown in Fig. 3. Files are organized into subfolders that combine subnumbers with species identifiers. Each of these subfolders contains two directories: anat and dwi. The anat directory includes T1- and T2-weighted images stored as "_T1w or _T2w.nii.gz" files. The dwi directory contains DTI data, comprising files with the extensions "_dwi.bval," "_dwi.bvec," and "_dwi.nii." Users can download the necessary datasets and perform high-resolution analyses, such as those shown in Figs. 4 and 5, using compatible software tools, including ImageJ, MRIcron, or DSI Studio. For detailed usage instructions and citation guidelines, please refer to the "README.txt" file included in the dataset.

Histological data deposit.   The histological data were obtained from the same individuals or those who had undergone MRI. Owing to the sampling procedure at the zoo, some brain slices could not be prepared because of the difficulty of placing them in the fixative solution immediately postmortem. Histological images, including H&E- and DAPI-stained specimens, have been uploaded to the SSBD[26].
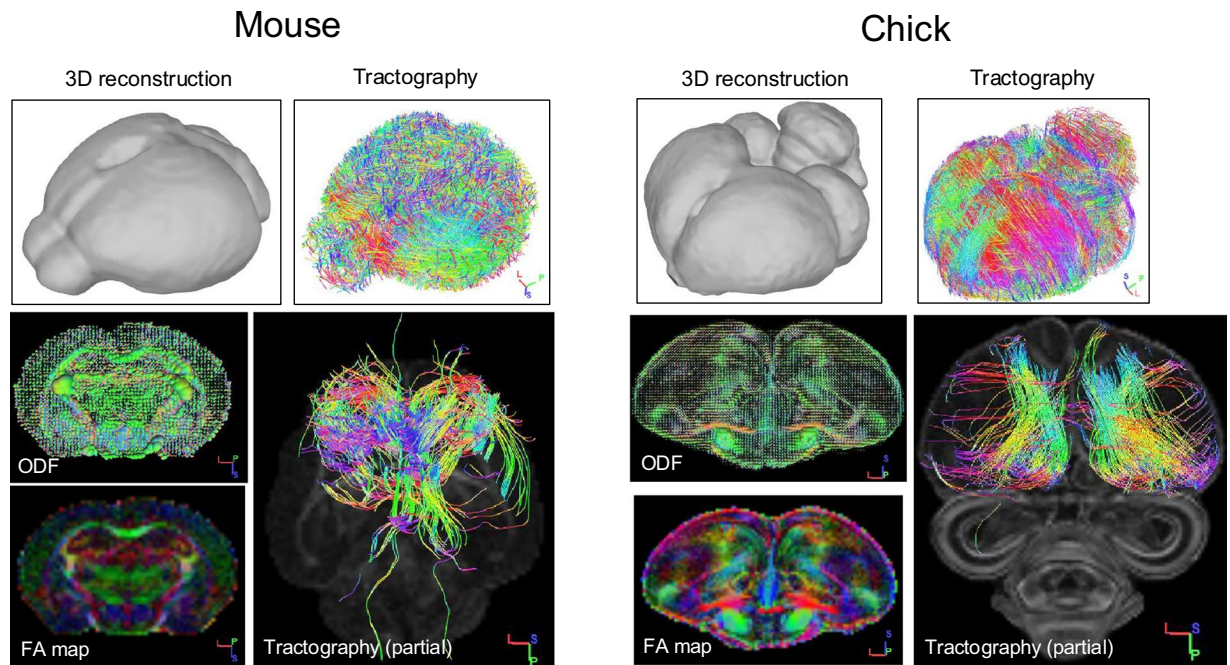
Fig. 5 Diffusion MRI-based analysis of structural connectivity in mouse and chick brains. Representative diffusion MRI outputs of the mouse (left) and chick (right) brains, including 3D reconstructions, whole-brain and partial tractography, ODFs, and fractional anisotropy (FA) maps. ODFs visualize the directional complexity of water diffusion, providing information on local fiber orientation, whereas FA maps indicate white matter integrity. Tractography results illustrate global and region-specific axonal pathways. All images were processed using DSI Studio.

Technical Validation

To ensure the quality, consistency, and reproducibility of the ABC dataset, we implemented several validation procedures throughout sample acquisition, imaging, and data processing.

Sample quality and preparation. All brain samples were obtained postmortem from collaborators, including zoos, universities, and research institutes, under appropriate MTAs. Samples were fixed in 4% paraformaldehyde and stored under standardized conditions. Before MRI scanning, samples were immersed in gadolinium-containing PBS to enhance signal contrast and Fluorinert to suppress background noise. This standardized preparation protocol helped minimize variability across samples.

MRI data validation. MRI data were acquired using 3 T and 7 T scanners depending on sample size. Scanning parameters were adapted from previously published protocols and optimized to maintain a signal-to-noise ratio > 20. Spatial resolution ranged from 100–200 μm for anatomical and diffusion-weighted imaging. Images were manually inspected for artifacts (e.g., motion, signal dropout, or misregistration) and underwent quality checks to ensure sufficient resolution for accurate structural delineation. To verify DTI data, FA maps and orientation distribution functions (ODFs) were calculated using DSI Studio. FA and ODF outputs were evaluated across multiple brain regions to confirm the biological plausibility of white matter tract organization, as illustrated in Fig. 5. These analyses also revealed species-specific patterns of commissural fiber connectivity (Fig. 4), serving as internal controls for anatomical validity.

Histological data validation. Whenever possible, histological sections were obtained from the same individuals used for MRI and prepared with standard H&E and DAPI staining procedures. Each section was visually inspected to confirm tissue preservation, appropriate staining, and alignment with the corresponding MRI slices. High-quality images were included in the SSBD repository.

Cross-modal consistency. We assessed anatomical consistency across imaging modalities by comparing coronal slices from T2-weighted MRI and histological staining to the extent possible. Major brain structures, including the cortex and commissures, showed good correspondence between modalities. This dual-view approach strengthens confidence in anatomical localization and enables multi-scale analysis within individual specimens.

Data reproducibility and accessibility. All imaging protocols and processing parameters are described in detail in the Methods section to support reproducibility. Raw data have been deposited in publicly accessible
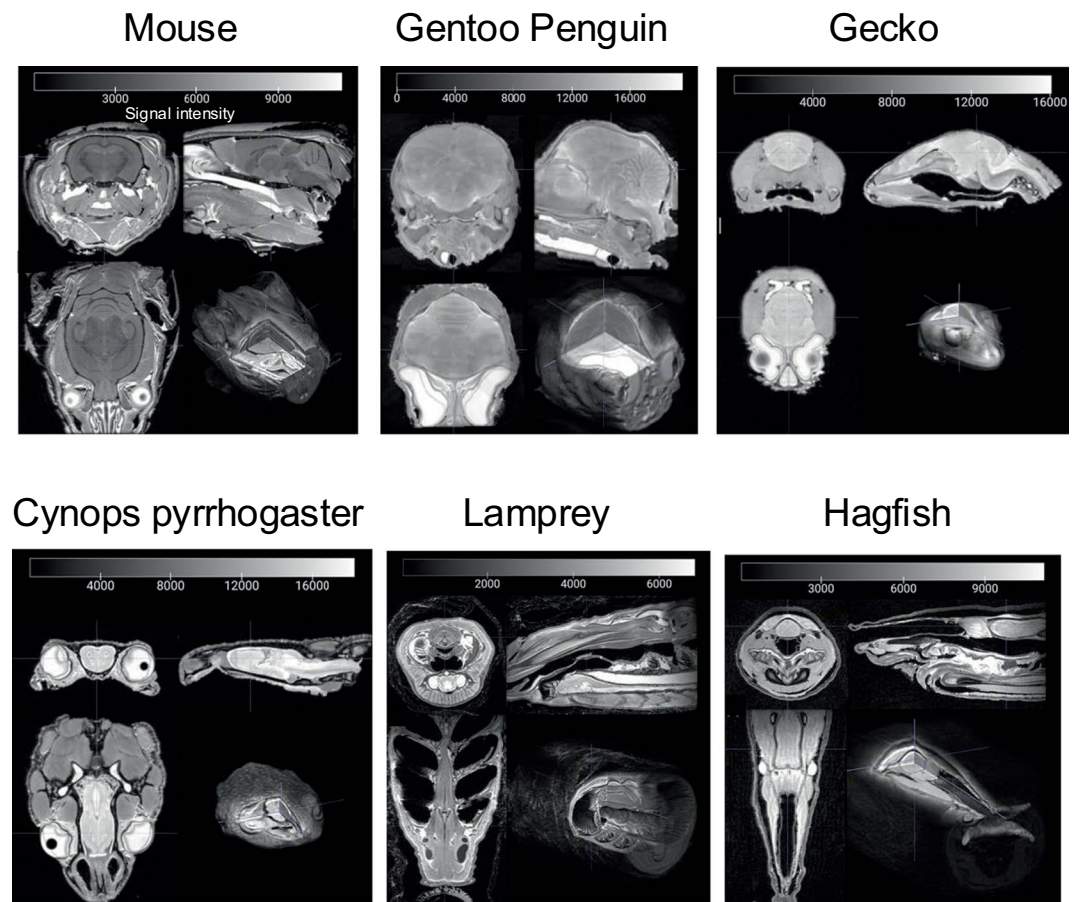
Fig. 6 Anatomical diversity across vertebrate species is showcased in the Animal Brain Collection. T2-weighted MR images in coronal and/or horizontal planes illustrate interspecies variation in brain morphology across six representative species: lamprey, hagfish, gecko, Japanese fire belly newt (Cynops pyrrhogaster), mouse, and gentoo penguin. These examples span a broad phylogenetic range, from jawless vertebrates to reptiles, amphibians, birds, and mammals, highlighting differences in brain size, shape, and internal architecture.

repositories (OpenNeuro and SSBD) in standard formats (e.g., NIfTI and TIFF). Users are encouraged to download and analyze the datasets using widely available software, such as MRIcron, DSI Studio, or ImageJ. Metadata accompanying each sample (e.g., species, developmental stage, and scan parameters) further enhance reproducibility and secondary analyses.

Usage Notes

In the context of database applications, ABC can serve as a valuable tool in the fields of neuroscience and evolutionary biology. Furthermore, brain morphology can be derived from the T1/T2 images in species with relatively unknown brain structures (Fig. 6). The advantage of MRI is that the brain structure can be explored with 3D viewer at moderate resolution. In the current database, which we acquired the T1 or T2 MRI images between 100 and 200 μm resolution, shows the distinct structure of white matter and gray matter in each species, birds, reptiles, and mammals, in 3D. Unlike traditional tissue samples, which only show the structure in isolated sections, MRI enables the observation of the entire brain, offering a significant advantage over traditional tissue samples. In addition to T1/T2 images, DTI images can also provide information on the behavior of fiber elongation and the connectivity of the white matter in certain brain regions (Fig. 4). For example, the corpus callosum can be identified in the mammalian Egyptian rousette bat and rabbit, whereas only the anterior commissure is acquired in the bird toco toucan and large-billed crow (Fig. 4). Additionally, we can compare the topographic mapping of the brain combined with a histological image, which would facilitate the creation of simpler images to connect the missing brain regions (Fig. 5). The structural connectivity shows the different property of brain-wide network among the species. The ODF, which is calculated from the diffusion-weighted MR images, is used to describe the directionality of multimodal diffusion in regions with complex fiber architecture present in brain and other biological tissues[27]. The distribution of ODF is quite different between mouse and chick.

Thus, the ABC database offers an accessible and expandable resource for exploring vertebrate brain structure and evolution, with potential integration of transcriptomic and genetic datasets in future updates. The dataset described here is also accessible via a public web interface, which allows interactive viewing of three-dimensional brain reconstructions derived from MRI scans, enabling cross-species comparisons.

Code availability

This study did not utilize any custom-written codes. All data processing operations followed the manuals and protocols of the relevant software. Specific parameters for each software and tool are detailed in the Methods section. In instances where detailed parameters were not specified, default parameters were used.

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Author contributions

T.T., Y.K. and T.K. designed the research. Y.K. acquired MRI images. T.T. performed MRI image analysis. T.K. performed histological image acquisition. I.A. managed the ABC website and uploaded the raw data to public databases. T.T., Y.K. and T.K. wrote the article. M.S. and C.O-M. revised the article.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to T.K.

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