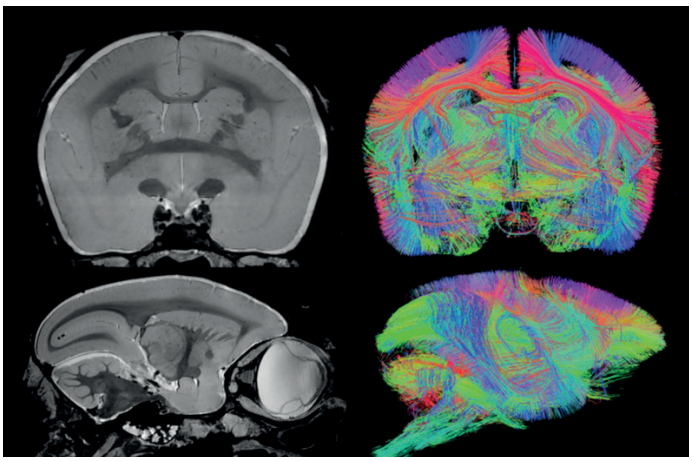

Non-Human Primate MRI Applications

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Many of today's fundamental biomedical questions can best be answered with the help of preclinical research. In comparison to other mammals, non-human primates (NHP) are characterized by a highly developed visual system, differentiated fine motor control and highly developed cognitive abilities. These characteristics make them ideal models for studying human capacities. Furthermore, the close proximity to human biology and cognition enables progressive research in the fields of cardiac diseases, infectious diseases, toxicity testing for medical substances, neuroscience, behavior and cognition, developmental studies, genetics, and xenotransplantation, all leading to advancements in the treatment and diagnosis of human physical and mental diseases. Current research with NHPs gives hope to the countless individuals suffering from cancer, diabetes, infectious diseases, acquired immunodeficiency syndrome (AIDS), cystic fibrosis, hepatitis, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and various dysfunctions of the brain.

Preclinical magnetic resonance imaging (MRI) enables exclusive non-invasive insights into living animals. This application note reviews exemplary research work completed on Bruker MRI instruments in the fields of developmental biology, neurology and cardiology in various NHP species.

Preclinical MRI Solutions for NHP Imaging

In general, the aforementioned studies are very demanding in terms of imaging hardware because they push the boundaries of what is currently feasible, to gain new research insights. It is therefore vital to use cutting-edge equipment in order to maximize the outputs and in turn limit the total number of NHPs to be investigated.

Bruker MRI systems offer superior performance for translational research on NHPs with large bore, high-field magnets, dedicated fast and strong imaging gradients and application optimized radio frequency (RF) coils. Large bore high-field magnets with free accessible bore sizes of 30 or 40 cm, as well as field strengths of 4.7, 7 or 9.4 Tesla are available. This enables studies of large animals with a high signal-to-noise ratio (SNR). Gradient strengths of up to 300 mT/m guarantee the highest temporal and spatial resolution, while gradient inserts can boost the gradient strength up to 1000 mT/m for smaller specimens or excised organs. A full portfolio of ^{1}H and ^{1}H/X volume and surface coils, multi-channel coils, and even custom-built RF coils for various application areas are available to ensure maximum performance.

ParaVision® is the industry standard in preclinical imaging software, with a full range of imaging sequences including ultra-short/zero echo time imaging (UTE/ZTE) and self-gated cardiac imaging (IntraGate™). This enables straightforward morphological and functional imaging of the entire anatomy, including organs that are challenging to visualize in MRI, such as the lungs and the beating heart.

Deciphering the Networks of the Brain

A deeper understanding of the physiology of cognitive processes and their neuronal correlates in the brain is mandatory to advance cognitive neuroscience. While most NHP experiments are aimed at understanding basic essential phenomena, the results also play a decisive role in the understanding of neurological disorders, leading to the development of new therapeutic methods.

Auditory and visual sensory pathways in NHPs are used to investigate where sensory information is encoded in the brain, how it is represented by neuronal activity, how this information is processed in the brain and finally how this representation is shaped by learning. The focus on visual perception and its modulation by cognitive factors, such as attention, aims to help those suffering from dysfunction in the brain's cognitive performance or visual reception, leading to disorders of perception and awareness.

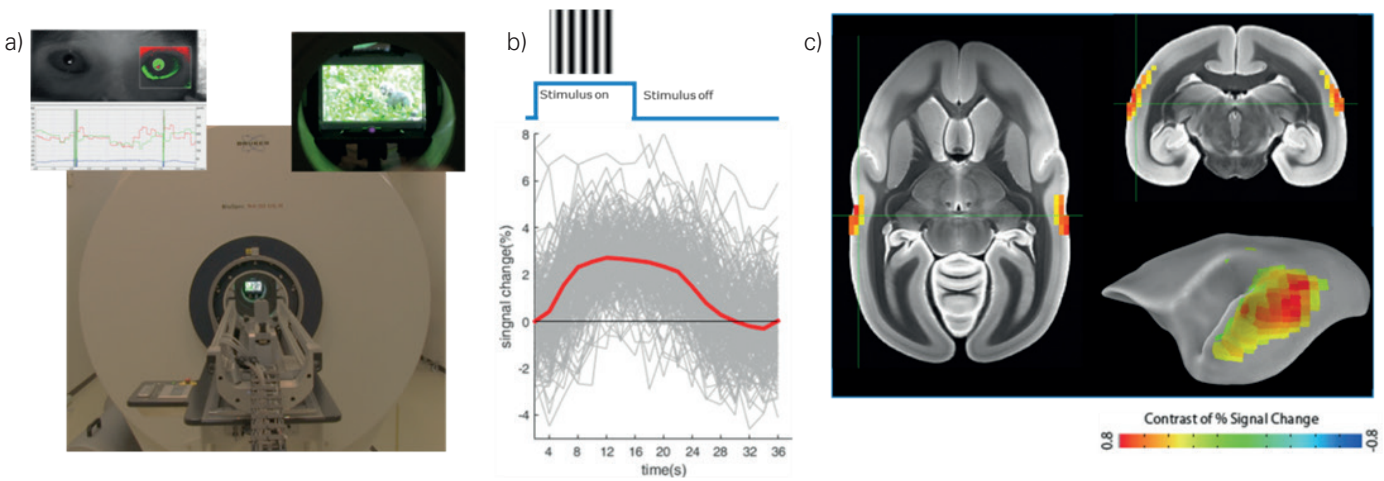
To resolve the functional architecture of the primate brain, functional Magnetic Resonance Imaging (fMRI) can be applied as demonstrated in Figure 1. Eye tracking as a behavioral measure was employed along with high-resolution fMRI to improve the understanding of the activity and function of complex neural networks in the marmoset brain and to better understand function homology between marmosets and humans.

The experiments clearly demonstrate that neural areas involved in perception of visual motion during visual stimuli are located in the middle temporal area, extending to the anterior part of the temporal cortex along the supra temporal sulcus.

Due to NHPs' highly developed visual capabilities, stimulation of the brain is often performed visually as in the aforementioned study. Understanding this pathway in the brain is therefore paramount to understanding all further neural connectivity. Figure 2 demonstrates the structural connectivity of the retina with the primary visual cortices via Diffusion Tensor Imaging (DTI) and tractography experiments, which were subsequently confirmed by Manganese Chloride injection into the retina, leading to hyperintense areas in which the $MnCl_2$ diffused through the optic nerve.

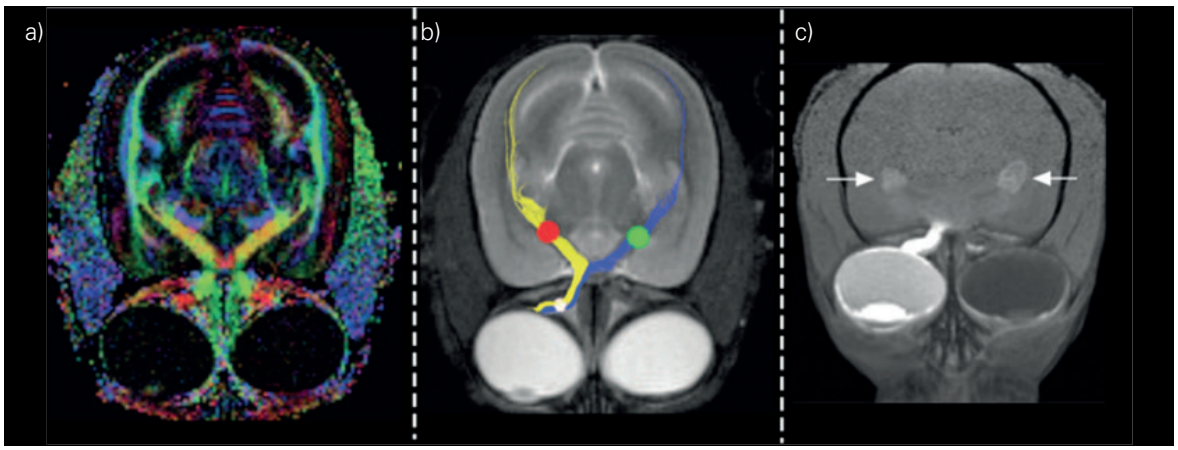
Brain function and functional connectivity are closely linked to the structural connectivity of the neurons. DTI and fiber tracts derived from DTI data based tractography deliver critical information about the connectivity between various brain regions. Diffusion imaging of the brain's structural connectivity requires strong diffusion weightings, which are made possible by strong MRI gradients. Structures in the NHP brain are much smaller than that of the human brain, so fast image acquisition methods are essential to deliver the fine resolution needed. Figure 3 demonstrates that, given the described prerequisites, even finest structures can be resolved in high resolution DTI and tractography images of NHP brains.

Figure 1



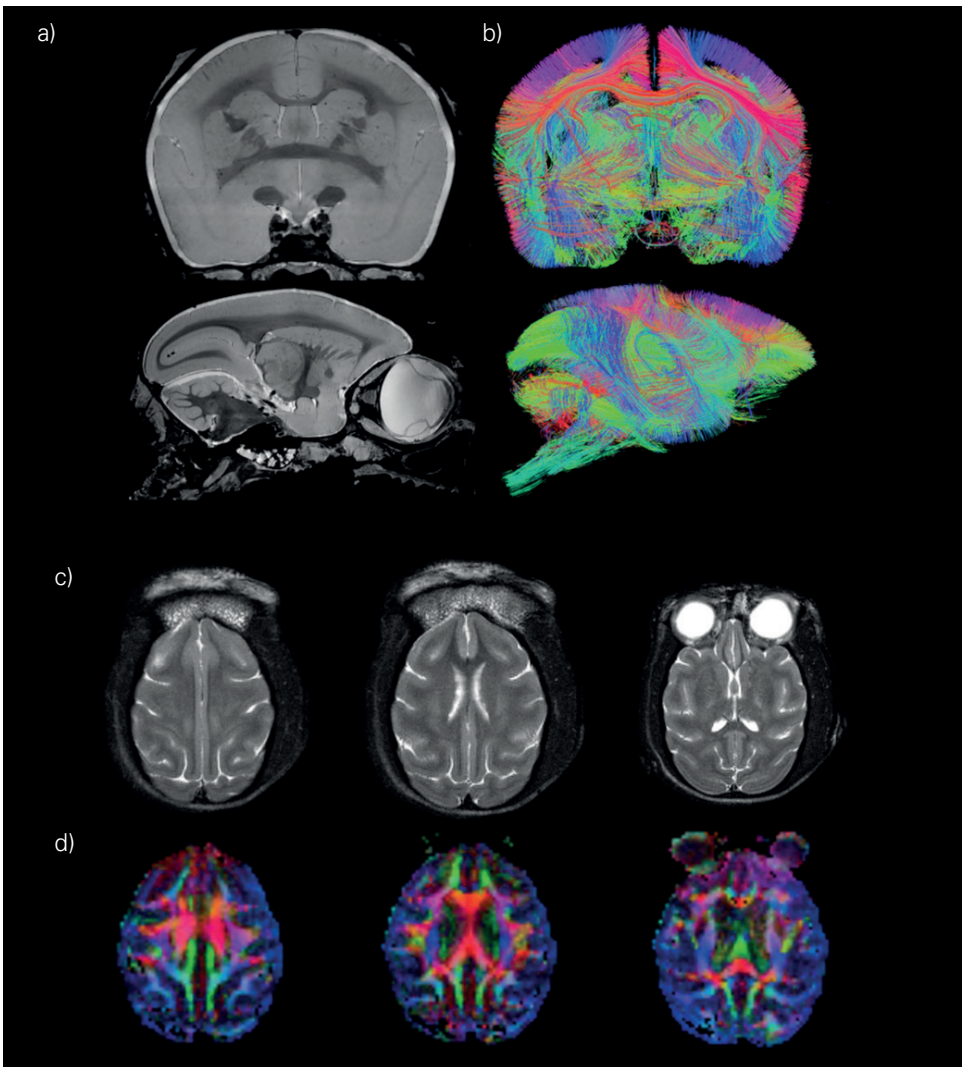
fMRI in awake marmosets at 9.4 T. a) + b) marmosets perform different cognitive tasks while inside the MRI instrument, resulting in signal changes due to the neural activity, as measured by the BOLD fMRI signal. A combination of visual stimulation and eye tracking software is used for the tasks. c) Neuronal architecture showing the visual motion sensitive area. Courtesy of T. Kaneko, Riken BSI, Wako, Japan.

Figure 2



DTI in NHP brain at 7 T; a) FA maps, b) Fiber tracking and c) Manganese enhanced MRI after retina injection with $MnCl_2$ to identify optic fibers. Courtesy of M. Yamada, H. Okano, et al., Keio University School of Medicine, Tokyo, Japan.

Figure 3



a) Highly resolved anatomical marmoset data; b) Corresponding DTI tractography data. Courtesy of J. Hata and H.Okano, Keio University, CIEA, Kanagawa, Japan.
c) Anatomical T2w data of non-human primate; d) Corresponding DTI FA maps. Courtesy of Wake Forest Baptist Medical Center, Winston-Salem, NC, USA.
All data was acquired at 7 T.

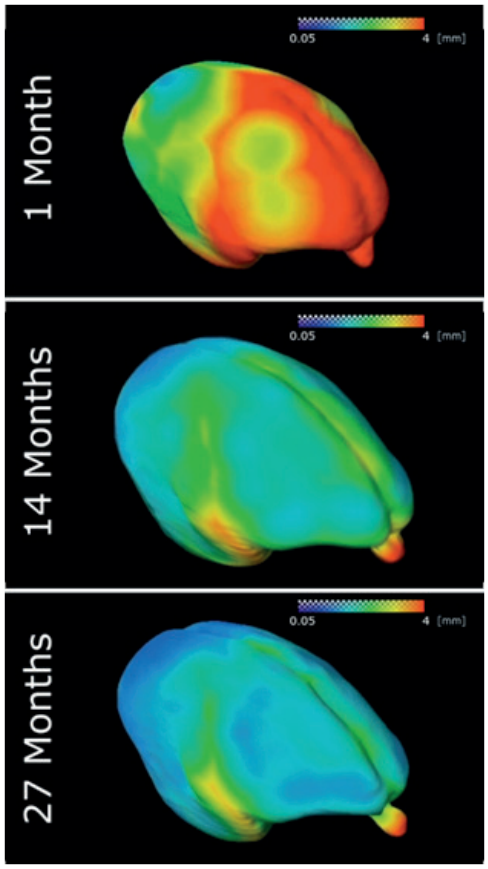
Developmental and Evolutionary Studies

In recent decades much developmental biology research has focused on a limited number of animal models. NHPs are predestined for this research area, due to their close evolutional proximity to humans translation of results to humans is all the more relevant.

Mapping brain development in the common marmoset enables neuro-developmental disease models to be evaluated precisely with transgenic techniques. Figure 4 demonstrates the cortical development derived from T_1w images taken over a period of more than two years, from adolescence to adulthood. Color-coded longitudinal changes of cortical thickness were derived. These demonstrate the morphological growth pattern in the marmoset brain, from the first month of development up to 27 months of age. After initial expansion, gradual thinning was observed, laterally and anteriorly in the brain.

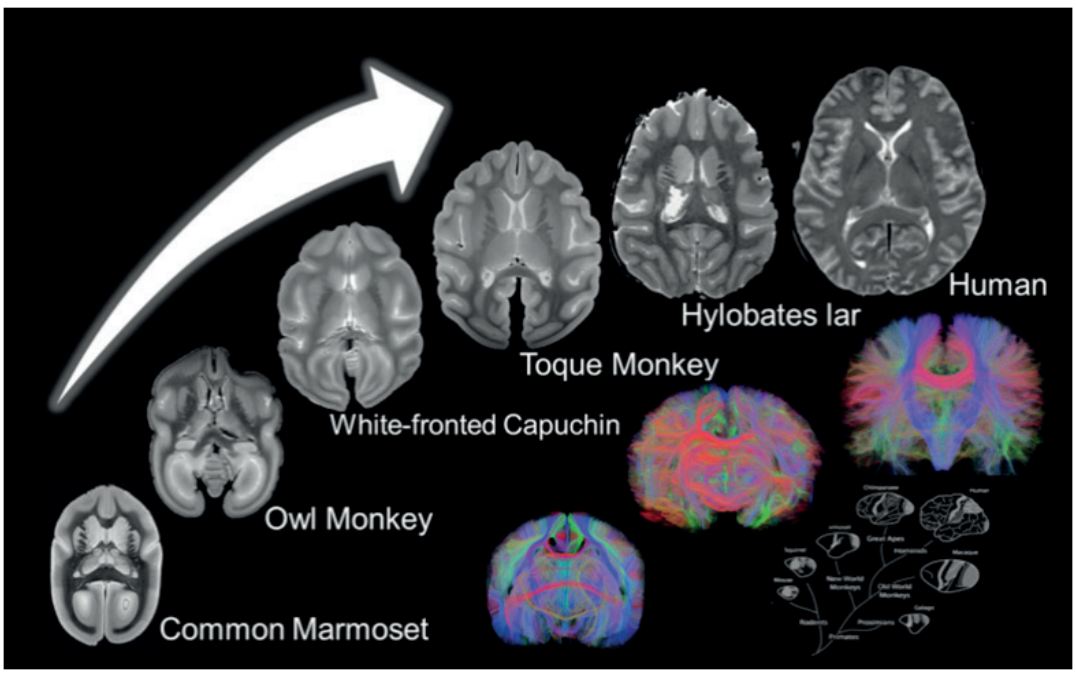
Evolutionary developmental biology is an additional field of biological research that compares the developmental processes of different beings to infer the ancestral relationships between them and to determine how developmental processes evolved. Figure 5 shows comparative neuroanatomical imaging in various NHPs and humans for elucidation of human brain evolution. T_2w images highlight evolutional changes in the cerebral sulcus. Combining these with fiber tracts derived from DTI data enables studies of the evolutionary development of neural network features and brain structure, from marmoset to man.

Figure 4



Brain development of marmosets from childhood to adulthood acquired at 7 T. The color bar indicates the cortical thickness. Courtesy of Ms. Seki, Dr. Kokamki and Prof. Okano, Department of Physiology, Keio University, Kanagawa, Japan.

Figure 5



Comparative neuroanatomical imaging of non-human primate brain samples measures at 9.4 T. Top row shows anatomical T_2w scans. Bottom row shows exemplary DTI scans. Courtesy of J. Hata, T. Sakai, and H. Ohta, Keio Univ., Kanagawa, Japan, Jikei Univ., Tokyo, Japan and Johns Hopkins Univ., Baltimore, USA.

Cardiac Studies

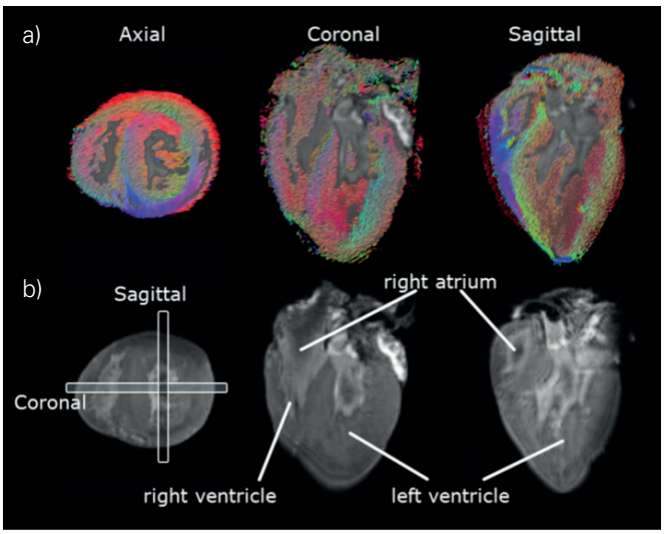
Heart disease is one of the most widespread and costly health problems facing mankind today. About 610,000 people die of heart disease in the United States every year, accounting for 1 of every 4 deaths.[1] On an individual level, the daily lives of patients who survive the disease are often severely restricted.

MRI is utilized in preclinical research for non-invasive assessment of the function and structure of the cardiovascular system of heart disease models. For *in vivo* imaging, ECG and respiration gating, navigator-based self-gated retrospective gating such as in IntraGate™ or radial short echo time imaging techniques such as UTE are used to compensate for heart motion and blood flow.

In addition to *in vivo* examinations, *ex vivo* heart measurements help to answer specific cardiological questions in a well-defined environment. Figure 6 shows the reconstruction of high resolution fiber tracts from DTI data in the marmoset heart *ex vivo*. This demonstrates that it is feasible to identify different heart tissues and microstructural changes in NHPs, for example during the progression of cardiovascular disease.

By combining a variety of cardiac MRI techniques into protocols, key functional and morphological features of the cardiovascular system can be assessed to study heart function, perfusion, blood flow, physiology, and micro-structure of disease models, with or without using contrast agents.

Figure 6



Ex vivo marmoset heart tractography at 7 T. a) Overlay of fiber tracts on the anatomical data. b) Anatomical images showing the different heart tissues. Courtesy of J.Hata and H.Okano, CIEA, Kanagawa, Japan.

Conclusion

MR imaging of NHPs is a well-established method for gaining insights into brain function, neuronal activation patterns and underlying structural connectivity, in the fields of developmental biology, evolutionary biology and cardiology. This is all the more important when research is urgently needed to help to understand the mechanisms of the onset and progression of many diseases and to finally find and test new therapeutic approaches for the benefit of mankind.

Bruker offers high performance preclinical MRI instruments for studying NHPs. Dedicated large-bore, high-field magnets in combination with state-of-the-art software and accessories address the needs of today's translational research.

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ALS	Amyotrophic Lateral Sclerosis
AD	Alzheimer's Disease
BOLD	Blood Oxygen Level Dependent
DTI	Diffusion Tensor Imaging
ECG	Electrocardiography
FA	Fractional Anisotropy
fMRI	Functional MRI
MRI	Magnetic Resonance Imaging
NHP	Non-Human Primate
RF	Radiofrequency
T_1w	T_1 weighted
T_2w	T_2 weighted
UTE	Ultra-short Echo Time imaging
ZTE	Zero Echo Time imaging

References

[1] CDC, NCHS. Underlying Cause of Death 1999-2013 on CDC WONDER Online Database, released 2015. Data are from the Multiple Cause of Death Files, 1999-2013, as compiled from data provided by the 57 vital statistics jurisdictions through the Vital Statistics Cooperative Program. Accessed Feb. 3, 2015.

[2] Tallon-Baudry C, Mandon S, Freiwald W, and Kreiter A. Oscillatory synchrony in the monkey temporal lobe correlates with performance in a visual short-term memory task. Cerebral Cortex 2004; 14:713–720.

[3] Langner S, Martin H, Terwee T, Koopmans S, Krüger P, Hosten N, Schmitz K, Guthoff R, and Stachs O. 7.1 T MRI to assess the anterior segment of the eye. Investigative Ophthalmology & Visual Science 2010; 51(12).

[4] Boretius S, Schmelting B, Watanabe T, Merkler D, Tammer R, Czeh B, Michaelis T, Frahm J, and Fuchs E. Monitoring of EAE onset and progression in the common marmoset monkey by sequential high- resolution 3D MRI. NMR in Biomedicine 2006; 19:41–49.

[5] Logothetis N. MR imaging in the non-human primate: studies of function and of dynamic connectivity. Current Opinion in Neurobiology 2003; 13:1–13

[6] Saleem K, Pauls J, Augath M, Trinath T, Prause B, Hashikawa T, and Logothetis N. MR imaging in the non-human primate: studies of function and of dynamic connectivity. Neuron; 34:685–700.

[7] Belcher A, Yen C, Stepp H, Gu H, Lu H, Yang Y, Silva A, and Stein E. Large-scale brain networks in the awake, truly resting marmoset monkey. The Journal of Neuroscience 2013; 33(42):16796–16804.

[8] Koyama M, Hasegawa I, Osada T, Adachi Y, Nakahara K, and Miyashita Y. Functional magnetic resonance imaging of macaque monkeys performing visually guided saccade tasks: comparison of cortical eye fields with humans. Neuron; 41:795–807.

[9] Hikishima K, Sawada K, Murayama A, Komaki Y, Kawai K, Sato N, Inoue T, Itoh T, Momoshima S, Iriki A, Okano H, Sasaki E, and Okano H. Atlas of the developing brain of the marmoset monkey constructed using magnetic resonance histology. Neuroscience 2013 Jan 29; 230:102-13.

[10] Sawada K, Sun X, Fukunishi K, Kashima M, Sakata-Haga H, Tokado H, Aoki I, and Fukui Y. Developments of sulcal pattern and subcortical structures of the forebrain in cynomolgus monkey fetuses: 7-tesla magnetic resonance imaging provides high reproducibility of gross structural changes. Brain Structure and Function 2009 Sep; 213(4-5):469-80.

[11] Hikishima K, Quallo M, Komaki Y, Yamada M, Kawai K, Momoshima S, Okano H, Sasaki E, Tamaoki N, Lemon R, Iriki A, and Okano H. Population-averaged standard template brain atlas for the common marmoset (Callithrix jacchus). Neuroimage 2011 Feb 14; 54(4):2741-9

[12] Okanoa H and Mitra P. Brain-mapping projects using the common marmoset. Neuroscience Research 2015; 93:3-7

[13] Duong T. Diffusion tensor and perfusion MRI of non-human primates. Methods March 2010; 50(3):125–135

[14] Bock N, Kocharyan A, Liu J, and Silva A. Visualizing the entire cortical myelination pattern in marmosets with magnetic resonance imaging. Journal of Neuroscience Methods 2009 December 15; 185(1):15–22

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