Magn Reson Med Sci 2019; 18; 253–259
doi:10.2463/mrms.mp.2018-0086
Published Online: February 7, 2019

MAJOR PAPER

# MR Imaging Properties of *ex vivo* Common Marmoset Brain after Formaldehyde Fixation

Yawara Haga[1,2,3], Junichi Hata[2,3,4*], Akiko Uematsu[2,3,4], Fumiko Seki[2,3,4], Yuji Komaki[2,4], Mai Mizumura[1,3], Marin Nishio[1,4], Takaaki Kaneko[2,3], Noriyuki Kishi[2,3], Hideyuki Okano[2,3], and Akira Furukawa[1]

**Purpose:** *Ex vivo* brains have different MRI properties than *in vivo* brains because of chemical changes caused by fixative solutions, which change the signal intensity and/or tissue contrast on MR images. In this study, we investigated and compared the MRI properties of *in vivo* and *ex vivo* brains.

**Methods:** Using a Bruker 9.4T experimental scanner unit for animals (Biospin GmbH, Ettlingen, Germany), we performed this study on the common marmoset. We measured the relaxation and diffusion values in the white matter and cortex of common marmosets and compared these values between *in vivo* brains ($n = 20$) and *ex vivo* brains ($n = 20$). Additionally, we observed the relationship between the tissue fixation duration and MRI properties by imaging a brain that underwent long-term fixation in a preliminary examination ($n = 1$).

**Results:** The $T_1$ values of *ex vivo* brains were decreased compared with those of *in vivo* brains; however, there were no significant difference in the $T_2$ and $T_2^*$ values of *in vivo and ex vivo* brains. Axial, radial, and mean diffusivity values of *ex vivo* brains decreased to approximately 65% and 52% of those of *in vivo* brains in the cortex and white matter, respectively. Conversely, fractional anisotropy values were not significantly different between *in vivo* and *ex vivo* brains.

**Conclusion:** The $T_1$ values and diffusion coefficient values of the *ex vivo* brains were strikingly different than those of the *in vivo* brains. Conversely, there were no significant changes in the $T_2$, $T_2^*$ or fractional anisotropy values. Altogether, the dehydration caused by tissue fixation and the reduction in brain temperature were involved in changing the relaxation and diffusion coefficient values. Here, it was difficult to specify all factors causing these changes. Further detailed study is needed to examine changes in MRI properties.

**Keywords:** *common marmoset, diffusion properties, postmortem magnetic resonance imaging, relaxation time*

## Introduction

Imaging brain specimens with MRI (i.e., *ex vivo* MRI) allows us to obtain higher-resolution images than those constrained by *in vivo* imaging because it enables image acquisition over periods as long as several days.[1] It is also useful in the study of brain anatomy because specimens can be subjected to section preparation for histological examination.[2]

*Ex vivo* brain MRI data are widely used in pathological and neurological studies.[3–6] For example, *ex vivo* brains have been used to evaluate changes in the white matter and/or volume changes in the hippocampus in Alzheimer's disease, to measure the volume of the frontal lobe gray matter and/or the lateral ventricles in schizophrenia, and to determine changes that occur in multiple sclerosis.[4,7–11] The *ex vivo* brain is also useful in forensic neurology research.[12]

Several previous MRI studies have measured physical values,[13] such as relaxation and diffusion values, that reflect tissue conditions for *in vivo* tissue assessment. Experiments to measure these values and compare them between *in vivo* and *ex vivo* brains have been conducted using the brains of mice, macaques, and humans.[14–23] Thus, the MRI properties of *ex vivo* brains have been examined using specimens from

[1]Department of Radiological Sciences, Human Health Sciences, Tokyo Metropolitan University Graduate School, Tokyo, Japan
[2]Department of Physiology, Keio University School of Medicine, Tokyo, Japan
[3]Laboratory for Marmoset Neural Architecture, Center for Brain Science, RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0106, Japan
[4]Live Imaging Center, Central Institute for Experimental Animals, Kanagawa, Japan
*Corresponding author, Phone: +81-48-462-1111, Fax: +81-48-462-1554, E-mail: j.hata@a3.keio.jp



Received: July 14, 2018 | Accepted: December 19, 2018

various animal species. However, there have been few studies with a statistically sufficient number of animals. In addition, among the various animals, there are not many studies that have compared measurements of these values between *in vivo* and *ex vivo* brains of the common marmoset (*Callithrix jacchus*). The common marmoset belongs to the primate group. The body length of an adult common marmoset measures up to 25 cm from the neck to the tail and they weigh approximately 350–450 g in captivity.[24–26] The common marmoset is useful as a psychiatric/neurological disease model because the pathology is similar to that in humans. Because of its short gestation period, it is also useful for tracking hereditary tendencies. Therefore, they have been used in recent neuroscience research.[27,28] In addition, neuroscience studies involving the common marmoset have used transgenic ($T_g$) common marmosets as subjects. $T_g$ mice have been conventionally selected for several studies, but this raises the issues of genetic and functional differences between mice and humans. The $T_g$ common marmoset is considered to solve this problem.[29,30] In fact, this animal was selected as a model in a Japanese national research project, Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS).[31,32] This project aims to establish a basis for elucidation of the structure and function of the human brain to help develop new treatments for psychiatric and neurological disorders using common marmosets as an animal model.[33,34]

In this study, we aimed to understand the MRI properties of *in vivo* and *ex vivo* brains of the common marmoset. We assessed differences in relaxation and diffusion values between *in vivo* and *ex vivo* brain MRI data. Furthermore, as a preliminary examination, we examined the relationships between fixation duration and MRI properties by imaging a brain that underwent long-term fixation ($n = 1$) as tissues of some specimens are often fixed and preserved for long periods of time.

## Materials and Methods

### *Animals*

This study was approved by the Animal Experiment Committees at the RIKEN Brain Science Institute and was conducted in accordance with the Guidelines for Conducting Animal Experiments of the RIKEN Brain Science Institute (H27-2-307).

Twenty healthy common marmosets (mean age, 6.0 ± 2.1 years; sex, 8 males and 12 females) and 20 *ex vivo* brains (fixed for 2.4 ± 0.9 days) were included in this experiment.

In a preliminary examination, the brain of a healthy 4-year-old male common marmoset was scanned (1 time *in vivo* and 11 times *ex vivo*).

### *Magnetic resonance imaging*

MRI was performed using a 9.4T BioSpec 94/30 (Biospin GmbH, Ettlingen, Germany) unit and a transmitting and receiving coil with an 86-mm inner diameter (40 mm for *ex vivo* brains). We obtained $T_1$, $T_2$, and $T_2^*$ mappings and diffusion-weighted images (DWI) from each animal. For $T_1$ mapping, rapid acquisition with relaxation enhancement was used with the following parameters: TR = 1200/1600/3200/4800/10000 ms, TE = 7 ms, flip angle = 90°, number of averages (NA) = 1, and scan time = 20 min. For $T_2$ mapping, a multiple spin-echo sequence was used with the following parameters: TR = 7000 ms, TE = 8/16/24/32/40/48 ms, flip angle = 90°, NA = 2, and scan time = 15 min. For $T_2^*$ mapping, a multiple gradient-echo sequence was used with the following parameters: TR = 2000 ms, TE = 3.5/8.5/13.5/18.5/23.5/28.5/33.5/38.5/43.5 ms, flip angle = 60°, NA = 2, and scan time = 10 min. The resolution was set at 270 × 270 × 540 μm in all cases. A partial coronal section perpendicular to the anterior comisure-posterior comisure (AC–PC) line and centered on PC was scanned in consideration of the limit of imaging setting. For DWI, spin-echo imaging and echo-planar imaging were used for the assessment of diffusion properties with the following parameters: TR = 3000 ms, TE = 25.57 ms, resolution = 350 × 350 × 700 μm, $\delta = 6$ ms, $\Delta = 12$ ms, $b$-value = 1000 s/mm$^2$ in 30 diffusion directions (plus 2 b0 images), NA = 3, and scan time = 30 min. Our *in vivo* and *ex vivo* brain experiments were performed under the same measurement conditions to permit accurate statistical analysis of the differences in relaxation and diffusion values between *in vivo* and *ex vivo* brains.

To acquire *in vivo* brain data, the animals were scanned in the supine position on an imaging stretcher and administered a mixture of oxygen and 1.5–2.5% concentrated isoflurane (Abbott Laboratories, Abbott Park, IL, USA). During the scan, heart rate, peripheral oxygen saturation ($SpO_2$), respiration, and rectal temperature were monitored regularly to manage the animal's physical condition. *Ex vivo* brains were also obtained by the same perfusion procedure but scanned after 2–3 days of fixation. To acquire *ex vivo* brain data, the brain was wrapped in a sponge and soaked in a fluorine solution, which exhibits no signal on MRI, in a plastic container. Vacuum degassing was performed to reduce air bubble-derived artifacts.

### *Data analysis*

The MRI properties in the current study were defined as physical values measured by mapping the relaxation time, i.e., $T_1$, $T_2$, and $T_2^*$ mapping, as well as tensor-based diffusion properties, i.e., fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). These properties were calculated with ParaVision 6.0.1 (Bruker, Inc., Ettlingen, Germany).

For image preprocessing, *in vivo* brain data were subjected to digital skull stripping (isolating the cerebral parenchyma) using Amira version 6.0 (Visage Imaging, Inc., San Diego, CA, USA). Since it was not possible to scan the whole brain with the maximum slice number at the shortest TR in the multi-slice method, the $T_1$-weighted images ($T_1$WI),

$T_2$-weighted images ($T_2$WI), and $T_2^*$-weighted images ($T_2^*$WI) were obtained by scanning a partial coronal section perpendicular to the AC–PC line and centered on PC. It is unified with all subjects and imaging dates. The diffusion images were registered to a "standard brain" image[27] using an Advanced Normalization Tools (ANTs) open-source software script.[35] Thus, the images were in the same space and the same ROIs could be used to generate the values of the MRI properties regardless of image acquisition point in space, day, or animal.

To assess the values of the MRI properties, ROIs were selected and drawn. The ROIs were 2-dimensional and drawn in the parasagittal cortex and white matter near the vertex (Fig. 1). We used hand-placed ROIs to prevent errors from including the margins of each region. We measured each value in both brain hemispheres and calculated the average. The images were measured in the coronal plane with ImageJ software version 1.5.1 (National Institutes of Health, Bethesda, MD, USA). The normality of the data for each value was confirmed using the Jarque–Bera test. The *in vivo* and *ex vivo* data were then statistically compared using a Student's *t*-test with Excel for Mac version 16.17 (Microsoft, Redmond, WA, USA). The level of significance was defined as $P < 0.05$.

## Results

The data from each set of 20 marmosets obtained by ROI analysis were used to draw box plots and examine differences between *in vivo* and *ex vivo* brains (Fig. 2). The $T_1$ relaxation values were significantly different between *in vivo* and *ex vivo* brains (Fig. 2a). A significant difference was also detected for the $T_2$ relaxation values in the white matter but not in the cortex (Fig. 2b). Additionally, a significant difference was detected for the $T_2^*$ relaxation values in the cortex but not in the white matter (Fig. 2c). AD, RD, and MD values were significantly different between *in vivo* and *ex vivo* brains (Fig. 2e–2g). In the cortex, the mean of the diffusion coefficient values from *ex vivo* brains were 65% of that from *in vivo* brains. In the white matter, the mean of the values from *ex vivo* brains were 52% of that from *in vivo* brains. There were no significant differences in FA values between *in vivo* and *ex vivo* brains in the two regions (Fig. 2d). Figure 3 shows a color map of the relaxation and diffusion values of *in vivo* and *ex vivo* brains. Figure 4 shows the relationship between the values and the postmortem duration in the preliminary examination plotted using a line graph. The $T_1$ values in these regions decreased remarkably within 3 days after tissue fixation and then showed a gradual decrease (Fig. 4a). The $T_2$ and $T_2^*$ values in these regions decreased remarkably within 1 week after tissue fixation and then showed a gradual decrease similar to the trend seen in $T_1$ values (Fig. 4b–4c). The AD, RD, and MD values in these regions showed a large decrease immediately after tissue fixation and remained approximately constant thereafter (Fig. 4e–4g). Regarding FA values, a significant change was not observed in these regions during the observation period (Fig. 4d).

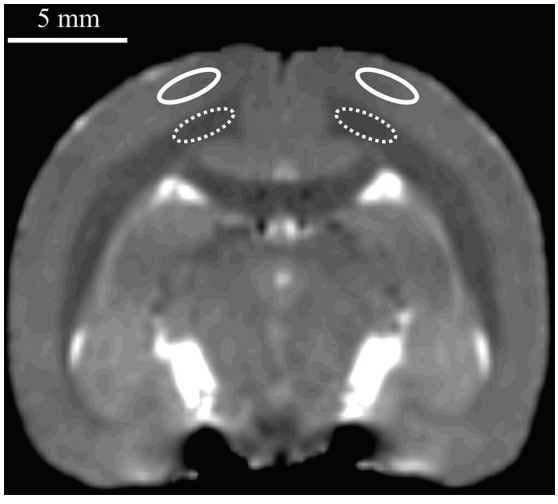


**Fig. 1** A diagram showing the ROIs drawn in the brain. This image shows the ROIs (circle: cortex, dotted circle: white matter) drawn for measurements.

## Discussion

Our data indicated that $T_1$ and the diffusion coefficient values greatly changed between *in vivo* and *ex vivo* brains while FA values were unchanged without regard for the brain state. The changes in relaxation values may be caused by the effects of tissue fixation and the reduction in brain temperature. The effects of tissue fixation were reported by Thavarajah et al.[36] in detail. The paraformaldehyde solution used in this study induces a cross-linking reaction between the functional groups of macromolecules such as proteins, which are the main components of brain tissue. The cross-linking caused by the tissue fixative solution occurs because of a chemical reaction. This maintains the protein and carbohydrate structures and prevents tissue autolysis and decay. At the same time, dehydration of the specimen tissue occurs. In addition, Birkl et al.[37] noted that the major reasons for the changes in the relaxation values were not only dehydration but also the decrease in brain temperature. They also showed that $T_1$ values were more affected by the change in brain temperature than were the $T_2$ and $T_2^*$ values. This tendency is consistent with the results from this study. In this study, the *in vivo* brain temperatures of the marmosets subjected to the experiment were speculated to be approximately 38°C, in reference to the study by Hayward and Baker.[38] In contrast, the *ex vivo* brain was maintained at room temperature at approximately 20°C during MRI scanning. Therefore, there was an approximately 20°C difference between the *in vivo* and *ex vivo* brains.

There is a high possibility that this temperature difference had a large effect on the relaxation value results. In summary, dehydration caused by tissue fixation and brain temperature reduction may have caused the reduction in relaxation values

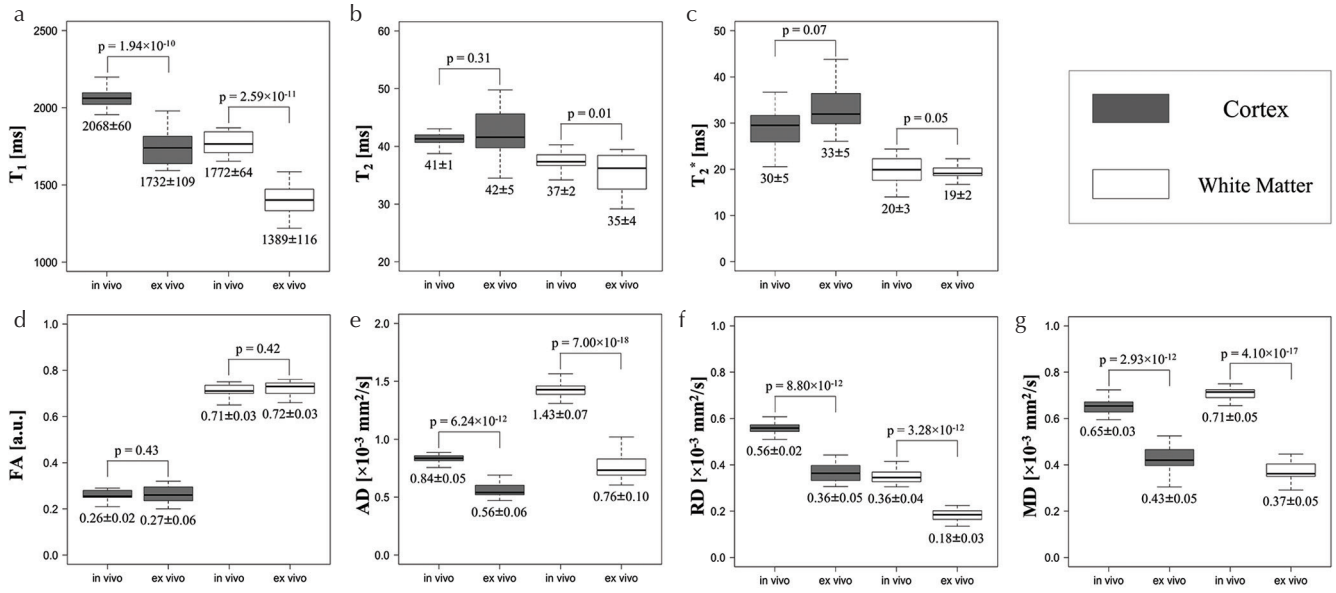


**Fig. 2** MRI measurement values from *in vivo* brains of 20 common marmosets and *ex vivo* brains from another set of 20 common marmosets. The top panel shows the tracking results of the changes in the relaxation values, i.e., $T_1$, $T_2$, and $T_2^*$ from the left. The bottom panel shows the results of FA, AD, RD, and MD from the left. The left and right halves of each figure show the values in the cortex and white matter (WM), respectively. The gray and white boxes represent the cortex and WM, respectively. The *P*-value listed at the top of each box plot was calculated by *t*-test. The mean and standard deviation are listed at the bottom of each box plot. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RA, radial diffusivity.

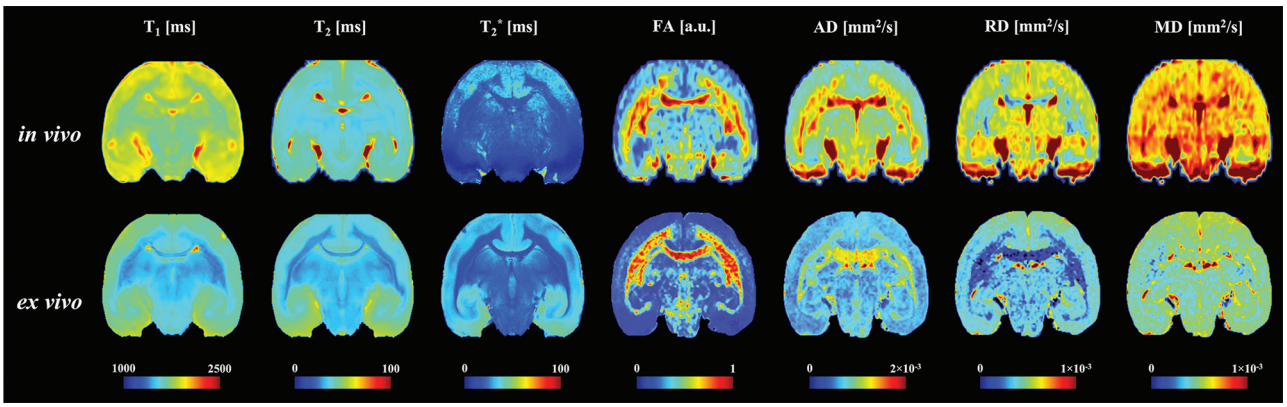


**Fig. 3** The difference of the relaxation values and the diffusion values of *in vivo* and *ex vivo* brain. The top panels show the results of the $T_1$, $T_2$, $T_2^*$, FA, AD, RD, and MD maps of an *in vivo* brain from the left. The bottom panels show the results of those maps of an *ex vivo* brain from the left. The color bar for each map is displayed at the bottom of each map image. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RA, radial diffusivity.

observed in our study. However, it is also clear that other factors affected the relaxation values and further examination is necessary in the future.

Research by D'Arceuil et al.[17] and Holz et al.[39] showed that decreasing brain temperature decreased diffusion coefficient values. Using the relational equation between the absolute temperature and diffusion coefficient derived by Holz et al.,[39] the diffusion coefficient value at 20°C is about 65.2% of the value at 38°C. The AD, RD, and MD values of the *ex vivo* brain were 66.7%, 64.3%, and 66.2% of those from the *in vivo* brain in the cortex and 53.1%, 50.0%, and 52.1% in the white matter, respectively. Accordingly, the differences in these values in the cortex were almost equal to the values calculated by the estimation equation shown in the previous study. On the contrary, in the white matter, the values in this study were about 10% lower than the values calculated by the estimation equation. Factors, other than dehydration, arising due to tissue fixation and temperature change may be involved in the difference between the cortex and white matter. However, we could not clarify the specific factors in this research and a more detailed examination is necessary.

On the contrary, our research showed that the FA values did not change after tissue fixation. This result is similar to the results shown in previous studies by D'Arceuil et al.[17]

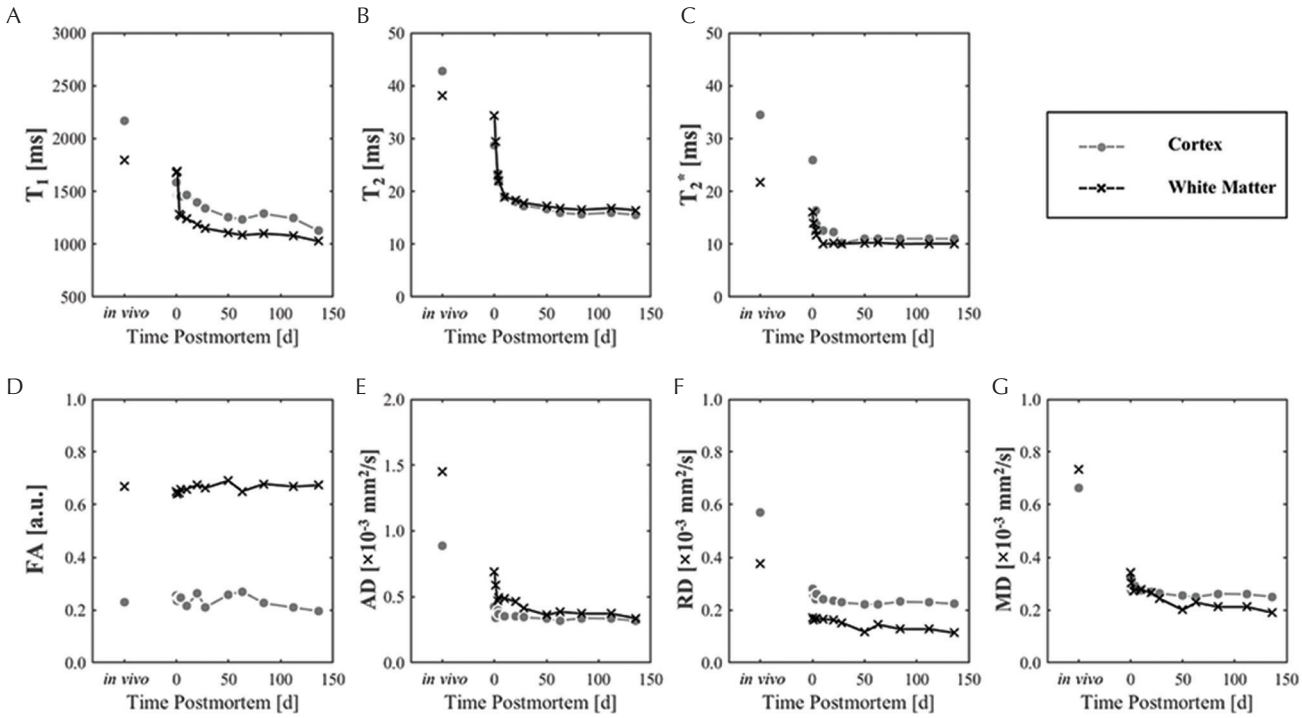


**Fig. 4** Long-term measurements of magnetic resonance imaging values in the *ex vivo* marmoset brain. The top panel shows changes in values related to relaxation properties, i.e., $T_1$, $T_2$, and $T_2^*$. The bottom panel shows FA, AD, RD, and MD results from the left. The curves in the graphs plotted with "circle" and "*x*" data points show the changes in the cortex and white matter, respectively. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RA, radial diffusivity.

and Guilfoyle et al.[18] The FA values are calculated from diffusion coefficient values in each direction ($\lambda$ = 1–3) and are unchanged when the values in all directions decrease by the same ratio. As described above, the rates of reduction in AD ($\lambda$ = 1) and RD (average values of $\lambda$ = 2 and 3) were almost the same in both regions. Therefore, there were no significant differences between the FA values of *in vivo* and *ex vivo* brains.

As a result of the long-term tissue fixation conducted in a preliminary study, we infer that structural MR images, such as $T_1$WI and $T_2$WI, will be nearly the same 1 week after tissue fixation. Similarly, it is obvious that the FA map can be obtained regardless of the tissue fixation duration. However, it should be noted that the diffusion coefficient values change significantly before and after tissue fixation treatment. Generally, tissue fixation is performed for about 2–3 days when performing a pathological assessment. However, in preclinical studies, we occasionally deal with tissue specimens that have been fixed for a long time. Therefore, experimental data from long-term fixed tissue serve as a reference for examination of MRI conditions and the interpretation of measured values when such specimens are imaged.

The limitations of this study need to be acknowledged. First, the brain temperature was not measured directly because of technical problems. In addition, in order to evaluate the factors causing changes in the relaxation and diffusion coefficient values in more detail, it is necessary to evaluate the existence of structural denaturation. However, *in vivo* micro imaging at the pathological level is difficult technically, so this examination could not be performed in this study.

A strength of our study is that we examined MRI properties using a statistically sufficient number of animals. However, additional research is required to clarify other factors that caused changes in each value. The next step would be to analyze similarities in nerve structures between *in vivo* and *ex vivo* brains. In this study, we showed that there were no significant differences between the FA values of *in vivo* and *ex vivo* brains. On the contrary, according to a previous study, the length and density of nerve fibers decreased with time after death.[40] Therefore, it will be important to look for differences in tractography results from *in vivo* and *ex vivo* brains to separate the influence of fixation time from that of diffusion time. If it is possible to evaluate neural structures equally in *in vivo* and *ex vivo* brains, a study on *ex vivo* brains with no limitation on the MRI acquisition duration would be highly useful.

## Conclusion

In this study, we measured relaxation and diffusion values and compared them between *in vivo* and *ex vivo* brains. The $T_1$ values of the *ex vivo* brains decreased to 80% of those of the *in vivo* brains. On the contrary, no significant changes in the $T_2$ or $T_2^*$ values were observed between the *in vivo* and

*ex vivo* brains. In addition, the diffusion coefficient values of the *in vivo* and *ex vivo* brains were significantly different in both the white matter and cortex regions. This decrease occurred at a roughly constant rate and the FA values were not significantly different between *in vivo* and *ex vivo* brains. We infer that the dehydration caused by tissue fixation and the reduction in brain temperature were related to the change in each value. However, since it is difficult to identify all factors affecting these changes, due to technical limitations, further studies are needed to investigate changes in MRI properties in greater detail.

## Acknowledgments

This research was partially supported by the program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from the Japan Agency for Medical Research and Development (AMED).

## Ethical Approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## References

1. Leprince Y, Schmitt B, Chaillou É, et al. Optimization of sample preparation for MRI of formaldehyde-fixed brains. Proceedings of the 23rd Annual Meeting of ISMRM, Toronto, 2015; 2283.
2. Dawe RJ, Bennett DA, Schneider JA, Vasireddi SK, Arfanakis K. Postmortem MRI of human brain hemispheres: $T_2$ relaxation times during formaldehyde fixation. Magn Reson Med 2009; 61:810–818.
3. Marshall VG, Bradley WG, Marshall CE, Bhoopat T, Rhodes RH. Deep white matter infarction: correlation of MR imaging and histopathologic findings. Radiology 1988; 167:517–522.
4. Moore GR, Leung E, MacKay AL, et al. A pathology-MRI study of the short-T2 component in formalin-fixed multiple sclerosis brain. Neurology 2000; 55:1506–1510.
5. Grafton ST, Sumi SM, Stimac GK, Alvord EC, Shaw CM, Nochlin D. Comparison of postmortem magnetic resonance imaging and neuropathologic findings in the cerebral white matter. Arch Neurol 1991; 48:293–298.
6. Pfefferbaum A, Sullivan EV, Adalsteinsson E, Garrick T, Harper C. Postmortem MR imaging of formalin-fixed human brain. Neuroimage 2004; 21:1585–1595.
7. Bronge L, Bogdanovic N, Wahlund LO. Postmortem MRI and histopathology of white matter changes in Alzheimer brains. A quantitative, comparative study. Dement Geriatr Cogn Disord 2002; 13:205–212.
8. Bobinski M, de Leon MJ, Wegiel J, et al. The histological validation of post mortem magnetic resonance imaging determined hippocampal volume in Alzheimer's disease. Neuroscience 2000; 95:721–725.
9. Selemon LD, Kleinman JE, Herman MM, Goldman-Rakic PS. Smaller frontal gray matter volume in postmortem schizophrenic brains. Am J Psychiatry 2002; 159:1983–1991.
10. Chance SA, Esiri MM, Crow TJ. Ventricular enlargement in schizophrenia: a primary change in the temporal lobe? Schizophr Res 2003; 62:123–131.
11. Seewann A, Kooi EJ, Roosendaal SD, et al. Postmortem verification of MS cortical lesion detection with 3D DIR. Neurology 2012; 78:302–308.
12. Yen K, Lövblad KO, Scheurer E, et al. Post-mortem forensic neuroimaging: correlation of MSCT and MRI findings with autopsy results. Forensic Sci Int 2007; 173:21–35.
13. Weiskopf N, Mohammadi S, Lutti A, Callaghan MF. Advances in MRI-based computational neuroanatomy: from morphometry to in vivo histology. Curr Opin Neurol 2015; 28:313–322.
14. Sun SW, Neil JJ, Liang HF, et al. Formalin fixation alters water diffusion coefficient magnitude but not anisotropy in infarcted brain. Magn Reson Med 2005; 53: 1447–1451.
15. Sun SW, Neil JJ, Song SK. Relative indices of water diffusion anisotropy are equivalent in live and formalin-fixed mouse brains. Magn Reson Med 2003; 50:743–748.
16. Rane S, Duong TQ. Comparison of *in vivo* and *ex vivo* diffusion tensor imaging in rhesus macaques at short and long diffusion times. Open Neuroimag J 2011; 5:172–178.
17. D'Arceuil HE, Westmoreland S, de Crespigny AJ. An approach to high resolution diffusion tensor imaging in fixed primate brain. Neuroimage 2007; 35:553–565.
18. Guilfoyle DN, Helpern JA, Lim KO. Diffusion tensor imaging in fixed brain tissue at 7.0 T. NMR Biomed 2003; 16:77–81.
19. Tovi M, Ericsson A. Measurements of T1 and T2 over time in formalin-fixed human whole-brain specimens. Acta Radiol 1992; 33:400–404.
20. Hikishima K, Quallo MM, Komaki Y, et al. Population-averaged standard template brain atlas for the common marmoset (*Callithrix jacchus*). Neuroimage 2011; 54:2741–2749.
21. Zhu J, Klarhfer M, Santini F, Scheffler K, Bieri O. Relaxation measurements in brain tissue at field strengths between 0.35T and 9.4T. Proceedings of the 22nd Annual Meeting of ISMRM, Milano, 2014; 3208.
22. de Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL. High magnetic field water and metabolite proton $T_1$ and $T_2$ relaxation in rat brain in vivo. Magn Reson Med 2006; 56:386–394.
23. Shatil AS, Uddin MN, Matsuda KM, Figley CR. Quantitative *ex vivo* MRI changes due to progressive formalin fixation in whole human brain specimens: longitudinal characterization of diffusion, relaxometry, and myelin water fraction measurements at 3T. Front Med (Lausanne) 2018; 5:31.
24. Schiel N, Souto A. The common marmoset: an overview of its natural history, ecology and behavior. Dev Neurobiol 2017; 77:244–262.

25. Araújo A, Arruda MF, Alencar AI, Albuquerque F, Nascimento MC, Yamamoto ME. Body weight of wild and captive common marmosets (*Callithrix jacchus*). Int J Primatol 2000; 21:317–324.
26. Tardif SD, Power ML, Ross CN, Rutherford JN. Body mass growth in common marmosets: toward a model of pediatric obesity. Am J Phys Anthropol 2013; 150: 21–28.
27. Hikishima K, Sawada K, Murayama AY, et al. Atlas of the developing brain of the marmoset monkey constructed using magnetic resonance histology. Neuroscience 2013; 230:102–113.
28. Okano H, Kishi N. Investigation of brain science and neurological/psychiatric disorders using genetically modified non-human primates. Curr Opin Neurobiol 2018; 50:1–6.
29. Sasaki E, Suemizu H, Shimada A, et al. Generation of transgenic non-human primates with germline transmission. Nature 2009; 459:523–527.
30. Park JE, Zhang XF, Choi SH, Okahara J, Sasaki E, Silva AC. Generation of transgenic marmosets expressing genetically encoded calcium indicators. Sci Rep 2016; 6:34931.
31. Okano H, Miyawaki A, Kasai K. Brain/MINDS: brain-mapping project in Japan. Philos Trans R Soc Lond B Biol Sci 2015; 370. pii: 20140310.
32. Okano H, Sasaki E, Yamamori T, et al. Brain/MINDS: a Japanese national brain project for marmoset neuroscience. Neuron 2016; 92:582–590.
33. Uematsu A, Hata J, Komaki Y, et al. Mapping orbitofrontal-limbic maturation in non-human primates: a longitudinal magnetic resonance imaging study. Neuroimage 2017; 163:55–67.
34. Seki F, Hikishima K, Komaki Y, et al. Developmental trajectories of macroanatomical structures in common marmoset brain. Neuroscience 2017; 364:143–156.
35. Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC. A reproducible evaluation of ANTs similarity metric performance in brain image registration. Neuroimage 2011; 54:2033–2044.
36. Thavarajah R, Mudimbaimannar VK, Elizabeth J, Rao UK, Ranganathan K. Chemical and physical basics of routine formaldehyde fixation. J Oral Maxillofac Pathol 2012; 16:400–405.
37. Birkl C, Langkammer C, Golob-Schwarzl N, et al. Effects of formalin fixation and temperature on MR relaxation times in the human brain. NMR Biomed 2016; 29:458–465.
38. Hayward JN, Baker MA. Role of cerebral arterial blood in the regulation of brain temperature in the monkey. Am J Physiol 1968; 215:389–403.
39. Holz M, Heil SR, Sacco A. Temperature-dependent self-diffusion coefficients of water and six selected molecular liquids for calibration in accurate $^{1}$H NMR PFG measurements. Phys Chem Chem Phys 2000; 2:4740–4742.
40. D'Arceuil H, de Crespigny A. The effects of brain tissue decomposition on diffusion tensor imaging and tractography. Neuroimage 2007; 36:64–68.