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3 shown in Fig. 1. All components of the stereotaxic apparatus consisted of  
4  
5 non-magnetic materials that fixed the head position of the rats during data  
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7 acquisition. The RF coil was designed to have an impedance of  $50 \Omega$  at a resonance  
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9 frequency of 127.76 MHz. An additional single-turn surface coil of 62 mm diameter  
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11 was also developed for better homogeneity, and was used for a single slice of  
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13 DSC-MRI. The RF power had to be reduced in these coils below that of the standard  
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15 human head coil because of the diameter of the small coil. The transmission signal  
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17 was therefore attenuated to 20 dB, which allowed the use of automated scanner  
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19 software including the calibration of the RF transmission power and receiver gains.  
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21 All rats were fixed on the stereotaxic apparatus. They were placed at the center of  
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23 the gantry and oriented with the cranio-caudal axis perpendicular to a static  
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25 magnetic field. Their heads were positioned inside the coil toward the cranio-caudal  
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27 direction.  
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38  $T_1W$  images were obtained with a conventional two-dimensional fast spin  
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40 echo (2D-FSE) sequence. The repetition time (TR) was 1500 ms [10]. The echo time  
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42 (TE) was fixed at 14 msec. The echo train length (ETL) was 3. The field of view (FOV)  
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44 was set at  $40 \times 30 \text{ mm}^2$ , the slice thickness at 1.5 mm, the slice gap at 0.5 mm, the  
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46 number of excitations (NEX) at 10, and the band width (BW) at 31.3 kHz. The  
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48 acquired matrix ( $256 \times 160$ ) was interpolated, and null pixels were added in k-space to  
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50 produce square matrices of  $256 \times 256$ . The acquisition time was 10 min 3 sec.  
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3 T<sub>2</sub>W images were obtained with a 2D-FSE and the following imaging  
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5 parameters: TR 4100 ms, TE 128 ms, ETL 14, FOV 40×30 mm, slice thickness 1.5  
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7 mm, slice gap 0.5 mm, NEX 8, BW 31.3 kHz, acquired matrix 256×160, zero-filled to  
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9 256×256, phase direction ventral-dorsal, and an acquisition time of 11 min 2 sec.  
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15 TOF-MRA was performed by use of a three-dimensional flow-compensated  
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17 spoiled gradient recalled (3D-SPGR) sequence prepared with magnetization transfer  
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19 and with TR 53 ms, TE 5.5 ms, flip angle (FA) 45 degrees, BW 16 kHz, FOV 8×6 cm,  
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21 slice thickness 0.2 mm, 1 acquired slab of 512×512×64, a voxel resolution 0.156 ×  
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23 0.156 × 0.2 mm<sup>3</sup>, NEX 1, and an acquisition time of 21 min 46 sec.  
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30 DSC images were obtained following the intravenous administration of  
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32 Gd-DTPA to the T<sub>2</sub>\*-weighted gradient echo dynamic images. A bolus of Gd-DTPA  
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34 (0.1 mmol/kg) was injected manually into the tail vein with a 22-gauge catheter via  
35  
36 one meter of polyethylene tubing (PE50, internal diameter: 0.58 mm / outer  
37  
38 diameter: 0.965 mm, Becton Dickinson and Company, Franklin Lakes, NJ, USA),  
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40 and was followed by an additional administration of saline (1.0 ml). A multi-shot EPI  
41  
42 with the number of shots at 2 was employed for improving EPI distortion and  
43  
44 temporal resolution. Imaging parameters were TR 142 ms, TE 22.1 ms, FA 20°, FOV  
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46 40×40 mm, and a matrix size of 64×64, leading to a pixel size of 0.625×0.625 mm<sup>2</sup>.  
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48 The slice thickness was 3 mm in single slice around the hypothalamus (HT). The  
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50 temporal resolution was 0.284 sec per image, and the acquisition time was 1 min 15  
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3 sec. This assessment was repeated three times at intervals of 40 min and 10 min,  
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5 corresponding to the 1<sup>st</sup>-2<sup>nd</sup> and 2<sup>nd</sup>-3<sup>rd</sup> scans, respectively.  
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### 8 9 10 *Data analysis*

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12 All MRI images were reconstructed on the same workstation provided for the  
13  
14 GE Signa 3-Tesla scanner as used for the clinical programs. Then the images were  
15  
16 transferred to a Linux workstation. Lastly, data analysis was carried out by use of  
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18 in-house and commercial software program.  
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23 To evaluate the T<sub>1</sub>W and T<sub>2</sub>W image quality, we calculated the  
24  
25 contrast-to-noise ratio (CNR) with an inter-tissue method [25-27] as follows:  $CNR =$   
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27  $(\pi/2)^{1/2} (SI_a - SI_b) / SI_{air}$ , where  $SI_{air}$  represent air-mean-signal-intensity,  $SI_a$  and  $SI_b$   
28  
29 represent the signal intensities of tissue  $a$  and tissue  $b$ , respectively.  
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34 Angiograms were created by generation of the partial maximum intensity  
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36 projection (MIP) with a commercial software programs (Virtual Place Liberty (VPL),  
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38 AZE Co. Ltd. Tokyo, Japan). Visible middle cerebral artery (MCA) branches and left  
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3 artifacts caused by air in the trachea, the area of the arterial circle of Willis was  
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5 excluded in the definition of the AIF. The anterior cerebral artery was also excluded,  
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7 because of possible susceptibility effects attributed to the venous blood. A Gaussian  
8  
9 filter of 1.1 mm full width at half maximum (FWHM) was applied to all dynamic  
10  
11 images. The time-versus-signal-intensity curves (TICs) were converted to the  
12  
13 Gd-DTPA concentration according to Eq. (1) given in the Appendix. Functional  
14  
15 mapping images of the mean transit time (MTT), cerebral blood volume (CBV), and  
16  
17 cerebral blood flow (CBF) were carried out with the deconvolution method [28]. The  
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19 theory is described in detail in the Appendix. For ROI analysis, images of  $64 \times 64$   
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21 matrix size were converted to  $256 \times 256$  with use of a sinc interpolation function.  
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### 33 Results

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35  $T_1W$  and  $T_2W$  images reconstructed with a spatial resolution of  $0.156 \times 0.188$   
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37  $\times 1.5$  mm are shown in Fig. 2. White matter could be discriminated from cortical and  
38  
39 deep-gray-matter regions. Locations of small anatomic features such as the caudate  
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41 putamen (CPu); striatum, the CC, and the hippocampus (HC) could also be identified  
42  
43 in both  $T_1W$  and  $T_2W$  anatomic images. The CNR between the HC and CC was 15.6  
44  
45 and 9.8, corresponding to the  $T_1W$  and  $T_2W$  images shown in Fig.2. The CNR  
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47 between the HC and IC was 23.2 and 13.6 respectively, in which underestimation  
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49 may exist in CNR attributed to the contamination of signal from the globus pallidus.  
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3 Results for MIP images obtained with contrast-free TOF-MRA are shown in  
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5 Fig. 3. Coronal MIP images around the HT of 5 mm thickness are shown in Fig.3 (A).  
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7 In this figure, the slice section contained ICAs and MCA. The MCA, the cortical  
8  
9 branches in both the left and right hemispheres, can be identified. It is important to  
10  
11 note that the anatomic structure of both cortical MCA arteries is different between  
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13 the right and left hemispheres. The ROI for the AIF was selected in the MCA region  
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15 indicated by the arrows in Fig.3 (A) and (B).  
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22 A typical example of a DSC-MRI image is shown in Fig.4. Distortion of the  
23  
24 DSC-MRI images is visible in Fig.4(A) toward the phase direction. The magnified  
25  
26 area in dynamic images around the MCA region, which is shown as a rectangle in  
27  
28 Fig.4(A), is displayed in Fig.4(B). There were several pixels which indicated temporal  
29  
30 changes in pixel contrast as a function of time, and these were reflected by Gd-DTPA  
31  
32 negative enhancement. The pixel signal intensity varied as shown in Fig.4(C), and  
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34 the curve shown was employed for estimating the AIF. Figure 5 shows the TIC in  
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36 this area together with the TIC for the whole brain region, obtained from each of the  
37  
38 three scans. The curves were visually reproducible in terms of shape, height and  
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40 width of the curves around the peak, as well as the tail height at end of the scan. It  
41  
42 should also be noted that the baseline before each injection of Gd-DTPA was  
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44 consistent, even though the 2<sup>nd</sup> and the 3<sup>rd</sup> curves should have been affected by the  
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46 previous injection of Gd-DTPA.  
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3           Functional mapping images of CBF, CBV, and MTT calculated according to  
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5 the theory described in the Appendix are shown in Figure 6. Images obtained from  
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7 this sequential assessment appeared to be reasonably clear, although slightly noisy,  
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9 and were consistent among the scans. The absolute CBF (mean±S.D.) in the  
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11 cortical-gray-matter area (mainly the parietal cortex (Pt)) was 24.04±2.88, 17.75±  
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13 3.34, and 31.87±7.27 ml/g/min, corresponding to the first, second, and third scans,  
14  
15 respectively. The Pt-to-HT CBF ratio was 46.7%, 51.5%, and 43.0%, corresponding to  
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17 the first, second, and third scans, respectively. The CBV was 0.49 (0.44), 0.50 (0.35),  
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19 and 0.47 (0.41) ml/ml in the Pt (the HT) region, corresponding to the first, second,  
20  
21 and third scans, respectively. The MTT in the same regions was 1.22 (2.51), 1.72  
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23 (2.29), and 0.92 (2.16) seconds, corresponding to each of the three scans. Among the  
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25 three injections, the absolute MTTs (mean±S.D.) were 1.29±0.40 sec in the Pt and  
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27 2.32±0.17sec in the HT region.  
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### 38 Discussion

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40           This study demonstrated that our system of a human whole-body 3-Tesla  
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42 MRI, fitted with an in-house solenoid coil developed for small animals, can provide  
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44 morphologic and functional images of the rat brain in vivo. The quality of T<sub>1</sub>-W and  
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46 T<sub>2</sub>-W images obtained with a scan duration of approximately 10 min was better  
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48 than those obtained in previous studies in which 1.5-Tesla clinical MRI scanners [3]  
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50 were employed. The neocortex and large subcortical structures, such as the Thal and  
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52 HC, are readily recognized in their topographic relationship to the CC, the  
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3 ventricular system, and the subarachnoid space [2]. In T<sub>1</sub>-W images, the  
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5 cerebrospinal fluid (CSF)-containing spaces are visible as hypo-intense, and the  
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7 arterial flow showed a signal loss caused by the so-called flow void effects, which is  
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9 typically seen in spin echo sequence [2]. In T<sub>2</sub>-W images, the CSF showed a bright  
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11 white intensity (prolonged T<sub>2</sub> relaxation time), whereas the myelinated white matter  
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13 showed black intensity (short T<sub>2</sub> relaxation time). The white-matter tracts such as  
14  
15 the IC and CC were clearly visible in both T<sub>1</sub>-W and T<sub>2</sub>-W images, with better quality  
16  
17 compared with previous reports employing 1.0- and 1.5-Tesla clinical MRI scanners  
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19 [2, 3].  
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28 MRA images also clearly showed the structure of distal MCA branches. The  
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30 MRA findings of inter-hemispheric differences with regards to MCA in the SD rat  
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32 were also consistent with a previous report on Wistar rats [29], which indicated the  
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34 left-to-right asymmetrical structure in three out of 10 Wistar rats using a 7-Tesla  
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36 MRI scanner dedicated to the small animal imaging [29]. Our MRA images are  
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38 superior to those in previous work [11] that employed a clinical 3-Tesla MRI scanner  
39  
40 and that only showed the major cerebral arteries and the carotid arteries because  
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42 they focused mainly on validating occlusion models [11]. The superior quality of our  
43  
44 MRA images can largely be attributed to the type of RF coil we used. Ours is a  
45  
46 three-turn solenoid coil that covers only the cerebral area, whereas the previous work  
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48 [11] employed a single-turn coil (diameter of 6.4 cm and length of 10 cm) that covered  
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50 the whole head, including the brain and the neck. Additionally, the prolonged  
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3 acquisition period in our study (almost twenty minutes) versus the significantly  
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5 shorter acquisition period in the previous study [11] (almost four minutes) may have  
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7 been a factor that led to higher-quality MRA images.  
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11 An important point with regard to this study is that DSC-MRI images of  
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13 reasonable quality can also be obtained with a clinical MRI scanner at 3 Teslas on  
14  
15 rats. In addition, we were able to extract the AIF from the rat brain, which is an  
16  
17 important accomplishment. Selection of an ROI in the MCA region successfully  
18  
19 provided the AIF. Spatial distortion or a susceptibility artifact was not visible in our  
20  
21 observation. Signal changes were obtained during DSC-MRI following a single dose  
22  
23 of Gd-DTPA. During this study, the dedicated transmitting and receiving RF coils  
24  
25 were considered essential for obtaining a reasonable SNR. Our study was performed  
26  
27 with the same sequence and the same dose rate (0.1 mmol/kg) of Gd-DTPA that are  
28  
29 commonly used in clinical examinations. Moreover, it was performed with high (1.17  
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31 mm<sup>3</sup>)-resolution dynamic imaging.  
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42 The quantitative images of CBF were consistent with a previous report on the  
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44 use of [<sup>14</sup>C] iodoantipyrine [30]. Namely, the Pt-to-HT contrast in CBF was 43-52 %  
45  
46 in this study, which is close to the values reported by Bloom et al. [30] of 44-58 %.  
47  
48 Although the absolute CBF and CBV values in our study were different from  
49  
50 previous ones [13, 30, 31], unsure scaling factors of each ones were canceled out in  
51  
52 calculating the MTT with Eq.(5) [32] (shown also in the Appendix). An analogous  
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54 value for the MTT was represented in previous work [31, 33]. In the theoretical  
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3 deconvolution step, the level of plasma gadolinium which was not influenced after  
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5 previous injection by calculated subtraction with peak to base line with  $(S(0)/S(t))$  of  
6  
7 Eq.(1) (Appendix).  
8  
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11  
12 We noticed that the absolute CBF and CBV values were overestimated,  
13  
14 which suggests that some limitations apply, such as the partial volume effect (PVE)  
15  
16 caused by insufficient spatial resolution as compared with the anatomic structure of  
17  
18 the MCA. Detection of AIF with repeat injection was performed (Fig.5, right row).  
19  
20 The major MCA diameter was approximately 0.5 mm at the maximum as evaluated  
21  
22 from Fig.3, which suggested that the measured AIF is largely influenced by the PVE  
23  
24 [24]. Also, the differences of absolute value may be reflected by the fact that a  
25  
26 non-linear relationship exists between the signal intensity and the contrast agent  
27  
28 concentration. Previous reports proposed methods for the non-linearity correction  
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30 for brain tissue [34] and AIF [35]. Further studies are needed to confirm the accuracy  
31  
32 and the reproducibility [36]. Image distortion caused by dielectric effects [37] and/or  
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34 EPI distortion [38] are also additional error sources, and should be investigated  
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36 systematically.  
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47 Along with the improved quality of acquired original dynamic images, the  
48  
49 mapping image quality would also improve. For better detection of the dynamic  
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51 susceptibility contrast that is caused by  $T_2^*$  signals, the optimization of TE, FA, and  
52  
53 the acquisition matrix should be investigated. We speculate that the multi-channel  
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55 phased array coil and parallel imaging techniques would reduce the level of  
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3 distortion. The DSC-MRI in this study was obtained only for a single slice. Further  
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5 careful attention is needed to multi-slice imaging in order to minimize the inflow  
6  
7 effects [35]. In our study, the contrast concentration  $C(t)$  curves including AIF's  
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9 varied slightly among the three injections. A sophisticated injector system which  
10  
11 need to be MR-compatible, may improve the variation.  
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18 A dedicated high-magnetic-field scanner equipped with a dedicated small  
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20 bore is the optimal device for small-animal imaging. However, such systems are not  
21  
22 commonly available. The system developed in this study might serve as a low-cost  
23  
24 solution or an alternative. The use of the present system provides an opportunity for  
25  
26 using the same imaging platform available for clinical studies for small-animal  
27  
28 imaging [7]. This would allow us understanding the pathophysiological status from  
29  
30 MRI signals using animal models with various diseases. More importantly,  
31  
32 optimizing several scan parameters which have been limited in clinical patients may  
33  
34 be performed easily on small animals with this system. In particular, the  
35  
36 reproducibility of CBF assessment with DSC-MRI, which has been reported the  
37  
38 limitation in clinical studies [19, 36], may be improved by performance of a  
39  
40 systematic evaluation of each scan parameter when this system is used on small  
41  
42 animals rather than clinical patients. With the addition of a high strength insert  
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44 gradient coil [39] which allow for thinner slices and much faster read-out, the system  
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46 performance can be enhanced and the spatial resolution with an acceptable SNR can  
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48 be improved. The use of adapting coils can be an effective solution for those who  
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3 operate MR scanners for human subjects and intend to gain experience [40] in  
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5 pre-clinical research.  
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## 8 9 10 **Conclusion**

11  
12 In this pre-clinical study on rats, reasonable image quality for morphologic  
13 information was obtained with T<sub>1</sub>-W, T<sub>2</sub>-W, and contrast-free TOF-MRA images,  
14 accomplished by use of a human whole-body 3-Tesla MRI scanner and newly  
15 developed solenoid coil. In DSC-MRI, it visualized transient signal changes with a  
16 single dose of Gd-DTPA, and with the same sequences which have been commonly  
17 used in clinical examinations. A human whole-body 3-Tesla MRI scanner and  
18 dedicated coil make it possible to detect the AIF in the MCA region of Wistar rats.  
19 High-resolution DSC-MRI was accomplished with a clinical scanner, but the spatial  
20 resolution with an acceptable SNR was insufficient for the rat brain. Although there  
21 might be some remaining issues with regard to AIF, we have shown the potential of  
22 DSC-MRI in our study.  
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## 42 **Appendix**

### 43 *Calculation of functional mapping images from DSC-MRI*

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45 The observed TIC  $S(t)$  was converted to a time-versus-concentration curve  
46 (TCC)  $C(t)$  by the following equation [16, 36]  
47

$$48 \quad C(t) = k \cdot \int R_2^*(t) = -k \cdot \ln(S(0)/S(t)) / TE, \quad (1)$$

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3 where  $\Delta R2^*$  is the change in the  $T_2^*$  relaxation rate and  $k$  is a constant. In this  
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5 study, it was assumed that  $k=1$ .  $S(0)$  is the precontrast (baseline) signal and  $S(t)$  is  
6  
7 the measured signal at time  $t$ . The next step was to fit this first-pass period of TCC to  
8  
9 a gamma-variate function;

$$10 \quad C(t) = a(t-b)^k \exp(-(t-b)/d), \quad (2)$$

11  
12 where  $a$ ,  $b$ ,  $c$ , and  $d$  were determined by a nonlinear least-square fitting. To minimize  
13  
14 the effects of the re-circulation of the contrast agent, data were neglected in the fit if  
15  
16 these concentrations were less than 50 % of the maximum after the peak of the TCC.  
17  
18 The fitted tissue TCC  $Ct(t)$  was deconvolved by the fitted AIF  $C_{AIF}(t)$  by use of a  
19  
20 singular value decomposition with a block-circulant deconvolution matrix (b-SVD)  
21  
22 method [28] and according to the equation

$$23 \quad CBF \cdot R(t) = Ct(t) \otimes^{-1} C_{AIF}(t), \quad (3)$$

24  
25 where  $\otimes^{-1}$  represents the deconvolution operator, and  $R(t)$  is a residue function  
26  
27 representing the tissue response to an instantaneous bolus.  $CBF \cdot R(t)$  was  
28  
29 estimated by deconvolving  $Ct(t)$  by  $C_{AIF}(t)$  using b-SVD, and then CBF was  
30  
31 determined as the maximum value of the obtained  $CBF \cdot R(t)$ .

32  
33 The CBV was calculated as follows:

$$34 \quad CBV = \int_0^{\infty} C_t(t) dt / \int_0^{\infty} C_{AIF}(t) dt. \quad (4)$$

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3 **Figure Captions**  
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7 **Figure 1**

8 The head fixation system for small animals, fitted with an MRI coil for RF  
9 transmission and reception. The coil is typically a three-turn solenoid; it can also be a  
10 single-turn coil, as shown on the top left of this figure.  
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16 **Figure 2**

17 T<sub>1</sub>-W images (top) and T<sub>2</sub>-W images (bottom) obtained from an SD rat, with the  
18 following anatomic locations as indicated: the somato-sensory cortex (SS), corpus  
19 callosum (CC), parietal cortex (Pt), external capsule (EC), caudate putamen (CPu),  
20 internal capsule (IC), hippocampus (HC), thalamus (Thal), auditory cortex (Au),  
21 amygdala (AM), hypothalamus (HT), internal carotid arterial system (ICAs),  
22 trigeminal nerve (TN), interpeduncular cistern (IPC), dorsal third ventricle (D3V),  
23 and the lateral ventricle (LV). These slice sections resemble the distance of -1.5 mm  
24 (left) and -3.5 mm (right), respectively, in appearance from the Bregma.  
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34 **Figure 3**

35 Partial maximum-intensity-projection maps of contrast-free time-of-flight MRA  
36 obtained for a Sprague-Dawley rat. Maps represent areas around the Thal (A), right  
37 hemisphere (B), and left hemisphere (C).  
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41 Note: the white arrows indicate the selected ROI level (as described in the  
42 Discussion). AZACA: Azygos anterior cerebral artery.  
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48 **Figure 4**

49 Typical time-frame images obtained from Gd-bolus tracking T<sub>2</sub>\*-weighted dynamic  
50 images (after the first injection). A white rectangle was placed in the MCA area (A),  
51 and its magnified images were visualized as (a), (b), (c), (d), and (e) in (B). TIC within  
52 the pixels is indicated by white arrows in (B), and the data were then plotted in a  
53 graph (C). Typical data points were extracted from the first injection at the times of  
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3 9.2 (a), 16.4 (b), 18.1 (c), 20.1 (d), and 33.9 (e) sec. In (A), the phase direction is shown  
4 as a white arrow.  
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8 **Figure 5**  
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10 TIC dynamic changes in the whole brain are shown in the first injection (A), the  
11 second injection (B), and the third injection (C). For each injection, a one-pixel ROI  
12 was selected and observed, as shown for the first (D), second (E) and third (F)  
13 injections, respectively. The negative enhancement changes in the rate from peak to  
14 base line on signal intensity were 16.8%(A), 10.0 %(B), and 10.3 %(C) at the whole  
15 brain (average 12.3, SD 3.9). The fraction of peak-to-base at selected ROIs were  
16 19.2 %(D), 14.5 %(E), and 16.9 %(F) (average 16.9, SD 2.3), for the first, second, and  
17 third injections, respectively.  
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27 **Figure 6**  
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29 CBF maps using the AIF from MCA, pictured in a gray scale from 0 to 4 ml/g/min, in  
30 the first column on the left. MTT maps are shown in the next column, followed by the  
31 CBV maps with 0 to 2 ml/g/min in the third and last column. Deconvolution was  
32 carried out pixel by pixel with b-SVD and then smoothed to  $0.15625 \times 0.15625$  mm  
33 in-plane resolution from acquired voxel resolution of a  $0.625 \times 0.625 \times 3$  mm ( $1.17$   
34  $\text{mm}^3$ ) in DSC-MRI.  
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Figure1  
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