Automated identification of piglet brain tissue from MRI images using Region-Based Convolutional Neural Networks

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Abstract

Magnetic Resonance Imaging is an important tool for characterizing volumetric changes of the piglet brain during development. These analyses have been aided by the development of piglet brain atlases which are based on averages drawn from multiple piglets. Because these atlases typically contain only brain tissue, their use is limited to "brain extracted" images from which the surrounding tissues have been removed. Brain extractions, or segmentations, are typically performed manually. This approach is time-intensive and can lead to variation between segmentations when multiple raters are used. Automated segmentations processes are important for reducing the time required for analyses and improving the uniformity of the segmentations. Here we demonstrate the use of region-based recurrent convolutional neural networks (RCNNs)

on a dataset consisting of 32 piglet brains. The RCNNs are trained from manual segmentations of sets of 27 piglets and then applied to sets of the remaining 5 piglets. The volumes of the machine-generated brain masks are highly correlated with those of the manually generated masks, and visual inspection of the segmentations show acceptable accuracy. These results demonstrate that neural networks provide a viable tool for the segmentation of piglet brains.

Introduction

Piglets are an important translational model for measuring the effect of nutrition on brain development. Not only do piglet brain stages of development correlate with human infant development, but their nutritional requirements are also comparable.(1) Magnetic resonance imaging (MRI) is an important technique for obtaining non-invasive measurements of brain volumes. A first step in volumetric analysis is the identification or "extraction" of the brain from the surrounding tissue. Manual segmentation has been the gold standard for brain extraction and is performed by creating an outline that separates the surrounding skull, muscles, tissues, and fat from the brain.(1-7) However, the method is not ideal when working with large data sets because it is time intensive and is subject to inconsistencies between raters/evaluators. Automated segmentation techniques are needed to overcome these limitations. In recent years deep learning has been shows to be a viable technique for medical image analysis and brain segmentation in animals and humans.(8-10) However, this process has not yet been applied to pigs. Here we demonstrate the use of region-based recurrent convolutional neural networks (RCNNs) for automated brain extractions of piglet brains.

Methods

Animals and Care Practices

All animal care and handling procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #18256) and were in accordance with federal guidelines. Male and female average-for-gestational age, Yorkshire crossbred, full-term, naturally-delivered piglets were obtained from University of Illinois Swine Farm at postnatal day 2 to allow for colostrum consumption. All piglets remained intact but did undergo routine processing on the farm including iron dextran (Henry Schein Animal Health, Dublin, OH, USA) and antibiotic injection (EXCEDE®, Zoetis, Parsippany, NJ 07054, USA) per routine farm practice and according to label. Four groups of piglets were placed individually into a caging system under standard conditions as described in a previous publication(11) and randomly assigned to five diet treatment groups. The control group of piglets remained with the sow until day 28 of age with free access to suckle and were weighed daily. The current study does not distinguish between diet groups.

Sow-raised piglets were handled daily, as were the artificially raised animals to diminish differences between the two types of rearing. After arrival at the Edward R. Madigan Laboratory (ERML) Animal Facility, experimental piglets received one dose of antibiotic: the first of two cohorts received Spectraguard™ (Bimeda, Inc, Oakbrook Terrace, IL 60181) on postnatal day 2 and the second cohort received Baytril® (Bayer healthcare LLC Shawnee Mission, KS 66201) on postnatal day 4. Additional doses of antibiotic were administered during the experiment only under the direction of the staff veterinarian. Animals were individually housed in racks of metabolism cages specifically designed to artificially rear neonatal piglets under constant 12-h light/dark

cycles. Piglet housing at ERML was as follows: space allowance for individual piglets was 30" deep, 23" wide, and 18.5" high, providing 4.8 square feet of floor space per piglet. Each piglet was supplied with a heating pad, toy, and blanket. Room temperatures were kept at 85-95 ºF using space heaters. Animals were also offered water or BlueLite® electrolyte solution ad libitum. Cages and heating pads were disinfected, and toys and blankets replaced daily. Animal care protocols were in accordance with National Institutes of Health Guidelines for Care and Use of Laboratory Animals and were approved by the University of Illinois Laboratory Animal Care and Use Committee.

MRI Acquisition

MRI data was acquired using 3 T Prisma scanner (Siemens, Erlangen) housed at the Biomedical Imaging Center at the University of Illinois. Pigs were anesthetized using TKX (combination of 2.5 ml of xylazine (100 mg/ml) and 2.5 ml of ketamine (100mg/ml) added to a Telazol vial and administered at a dosage of 0.02-0.03 ml/kg IM) then maintained on isofluorane (1-3%) throughout the imaging. Animals were scanned in the supine position using a specialized piglet head coil (Rapid Biomed, Rimpar). During scanning, the respiration rate, heart rate and blood oxygen levels were monitored using a LifeWindow LW9x monitor (Digicare, Boynton Beach, FL).

MRI Structural Imaging: Our structural MRI scan consisted of a MPRAGE acquisition (voxel size = 0.6 x 0.6 x 0.6 mm^3, FOV = 173 x 173 mm^2, 256 slices, GRAPPA - GeneRalized Autocalibrating Partial Parallel Acquisition - acceleration factor R = 2; TR = 2060 ms, TI = 1060 ms, flip angle = 9º, for an overall scan time of 5:21 min).

Manual brain extraction

Manual brain extraction was facilitated by first performing a rigid alignment of T_1-weighted images from all piglets to the brain atlas.(12) This was done using SPM12 imaging analysis software. First, we performed a manual rotation to approximately align the T_1-weighted images with 28-day piglet template(12), without resampling. We then used the "coregistration" function of SPM12 to further align the T1-weighted images to the template, again without resampling. The resulting alignment was accurate for all piglets, despite the fact that it was performed without first performing brain extraction, as shown by a representative image in Figure 1.

-------- Figure 1 about here ----------

Figure 1. Results of coregistration of piglet brain to the average brain template. The top row shows images from a representative piglet brain and bottom row shows the same slices from the average brain template. Blue lines within each image indicate the locations of the perpendicular slices. The consistency of two sets of images confirms good approximate alignment of the T1-weighted image with the average brain template.

An atlas-based brain mask was then resampled into the native space of each piglet, providing initial estimates of the brain masks for each piglet. These initial estimates were then modified to create individualized brain masks using Slicer3Ds segmentation editor, as shown in Figure 2. The majority of revisions were done in the sagittal plane, but all three orthogonal views were reviewed and modified for improved precision. All extractions were performed by one rater to minimize variability.

-------- Figure 2 about here ----------

Figure 2. Demonstration of the creation of manually-defined brain mask via modification of the template brain mask. A representative T1-weighted image is overlayed with the template brain mask, creating a brighter region. The green outline shows the manual-designated brain mask. The manual brain mask was created by editing the template brain mask for each individual piglet.

Automated brain extraction

Training was performed on 27 of the 32 piglets, with the remaining 5 used for evaluation. This process was then repeated using the same untrained model and a different train/evaluation subject split, thereby providing 10 piglets for validation analysis. Once training was completed, the evaluation subjects were fed into the model, where masks were generated for each slice based on the model's predictions.

This study uses a deep learning instance segmentation neural network using object detection model Mask-RCNN with Inception Resnet v2 architecture, pretrained on COCO 2017 data set(13). Our model uses a Tensor Flow 2.4.0 implementation of Mask R-CNN, with feature extractor Faster R-CNN (faster_rcnn_inception_resnet_v2_keras). Faster R-CNN utilizes a Region Proposal Network (RPN) backbone to generate the object proposals. The network then predicts the bounding box and object class for the proposed regions. Training was performed using a single NVIDIA GeForce GTX 1070 Ti GPU, with NVIDIA developer driver 465.21, CUDA 11.0, and CUDNN library 8.0.4.

The network was trained for 200,000 iterations with a cosine decay learning rate of 0.008 and a momentum optimizer value of 0.9. The training and segmentation were performed only in 2D sagittal planes. We threshold at confidence parameter 0.5.

Validation

Validation consisted of visual inspection of the machine-generated masks. We also performed Pearson correlations of the volumes of the manual masks with those of the machine-generated masks.

Results

Visual inspection of the brain extractions reveals good accuracy of automatic brain extractions (Figure 3). We quantified the consistency of the automated masks with the manual masks by comparing the brain volumes from the manual masks and machine-generated masks, denoted by V_{brain_manual} and $V_{brain_machine}$, respectively. The percent difference of the two volumes is given by $(V_{brain_machine} - V_{brain_man})/V_{brain_man} \times 100$. Percent difference values ranged from -9.6 to 3.0, as shown in Table 1. A Pearson correlation of V_{brain_manual} and $V_{brain_machine}$ on the combined data from both groups confirms good consistency, with R=0.70, p=0.03, N=10 (see Figure 4).

-------- Figure 3 about here ----------

Figure 3. Sample extractions from two subjects. The overlay that creates a brighter region indicates machine extractions, and the green outline shows the manual brain masks. The top row shows a piglet from model A, and the bottom row shows a piglet from model B.

The largest deviation of $V_{brain_machine}$ from V_{brain_manual} was -9.6% for the fifth pig of Group2. Visual inspection of the masks revealed that the manual segmentation included non-brain regions in the anterior portion of the brain near the olfactory bulb, presumably due to human error (see Figure 5). However, this error was not propagated to the machine segmentation, which conformed better to the brain. We conclude that in this case the automated extraction performed better than the manual extraction, confirming the value of an automated approach. When this pig was excluded, the correlation between V_{brain_manual} and $V_{brain_machine}$ exhibited improved consistency, R=0.93, p=0.0003, N=9.

-------- Figure 4 about here ----------

Figure 4. The consistency of brain volumes, V_{brain_manual} and $V_{brain_machine}$, from manual and machine segmentations, respectively. The solid line indicates unity and the colors denote the models used.

	V_{brain_manual} (cc)	$V_{brain_machine}$ (cc)	Percent difference
Group 1	70	71.2	1.7
	66.2	68.2	3.0
	64.4	62.9	-2.3
	64.5	63.5	-1.6
	67.6	67.1	-0.7
	70.2	69.2	-1.4

Group 2	60.8	61.5	1.2
	66.2	66.3	0.2
	64.9	65.8	1.4
	72	65.1	-9.6

Table 1. Brain volumes from manual and machine segmentations, V_{brain_manual} and $V_{brain_machine}$, respectively. Percent difference values are calculated according to $(V_{brain_machine} - V_{brain_man})/V_{brain_man} \times 100$.

-------- Figure 5 about here ----------

Figure 5. The green outline depicts a manually created mask that included non-brain regions as a result of human error. The brighter region shows the machine generated mask, that more successfully removed non-brain regions.

Discussion

We have shown that RCNNs trained on manually generated masks can be used to generate accurate brain masks. The use of automated brain extraction has the potential to reduce analysis time because it requires minimal supervision. This process is scalable to a high number of piglets, avoiding complications and inconsistencies that might arise from having multiple raters performing manual segmentations.

Improvements to our approach could be implemented in a variety of ways. Training with a larger sample size is expected to increase performance and accuracy of machine learning algorithms.(14)

Performance may also be improved by hyperparameter tuning and optimization.(15, 16) Piglet brain extractions might also be accomplished using 3D convolutional neural networks, a computationally intensive task that may be more accessible in the future. Further research is also needed to assess how machine learning may compliment or improve upon existing methods for segmenting brain sub-regions.(4, 17, 18) Of particular importance is the potential use of machine learning for creating age-dependent atlases of piglet development which could advance structural analysis of neonatal piglets.(3)

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Author contributions

KLS: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – Original Draft Preparation, Writing-Review & Editing **RJL:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing-Review & Editing **AL:** Investigation, Resources **BJL:** Conceptualization, Funding Acquisition, Investigation, Project Administration, Supervision, Writing-Review & Editing **LR:** Funding Acquisition, Investigation, Project Administration, Resources, Supervision **AS:** Funding Acquisition, Supervision, Writing-Review & Editing

Data Sharing

Processing scripts are available at https://github.com/kaylals2/Piglet_RCNN.

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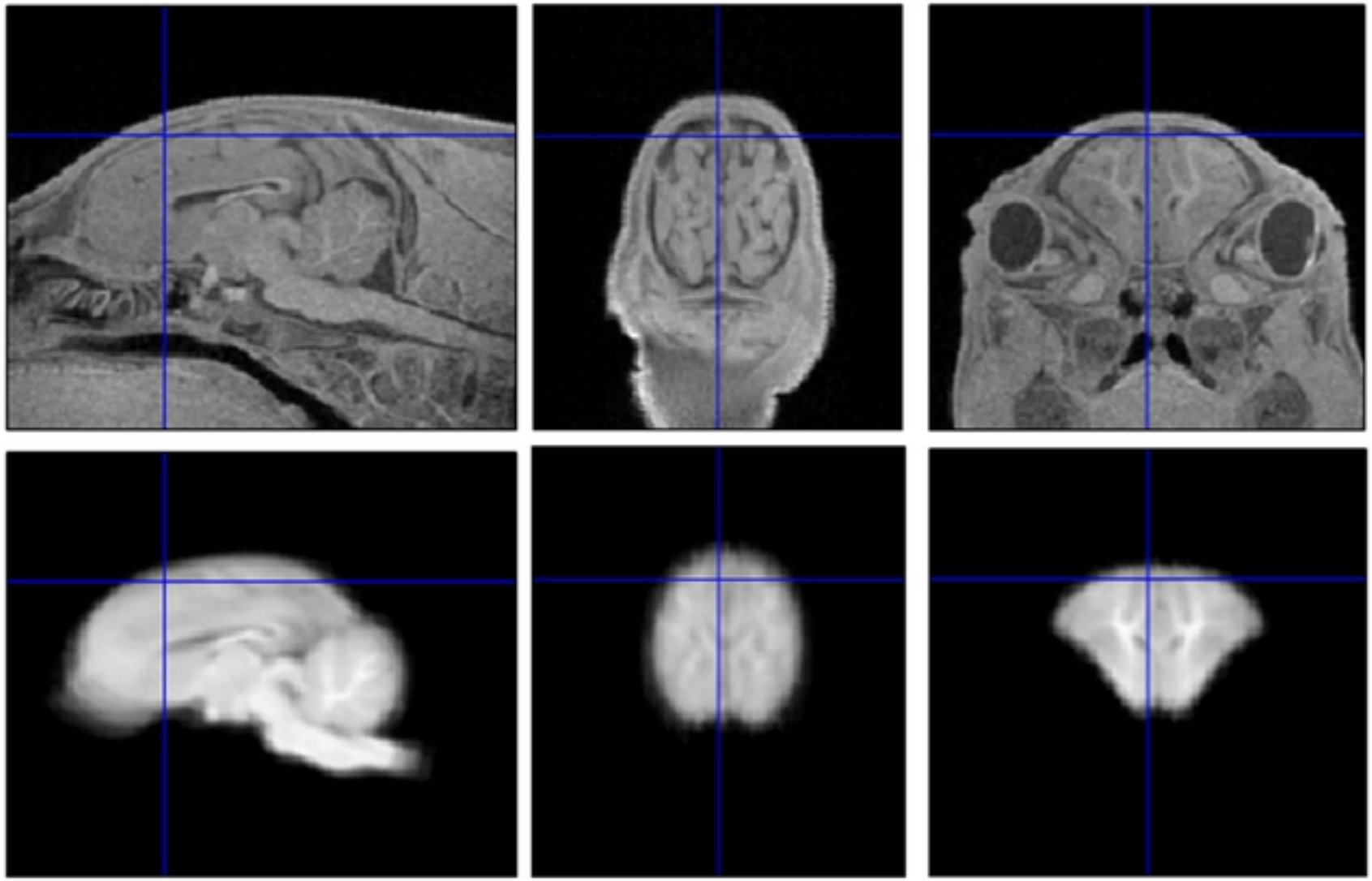

Figure 1

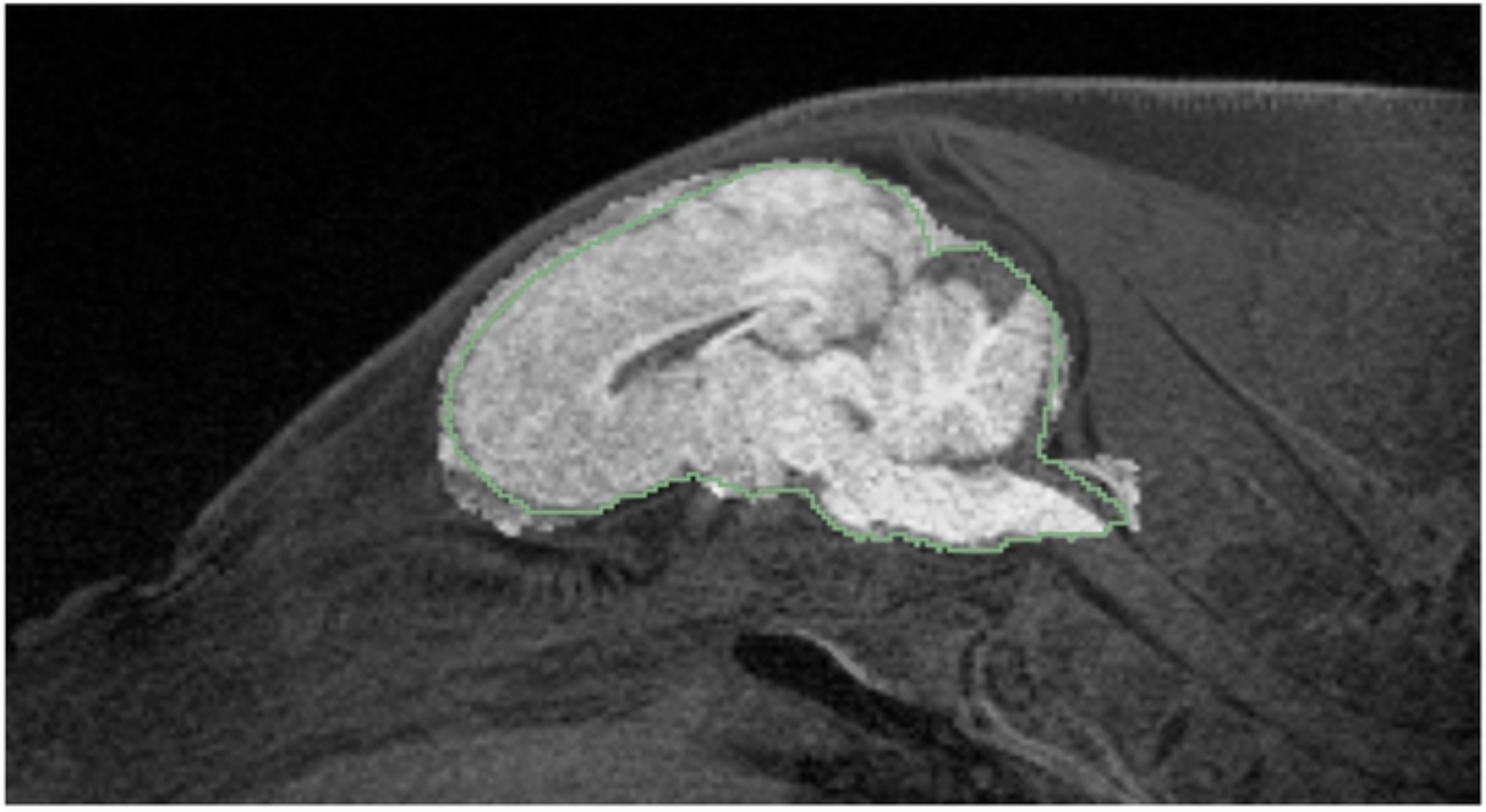

Figure 2

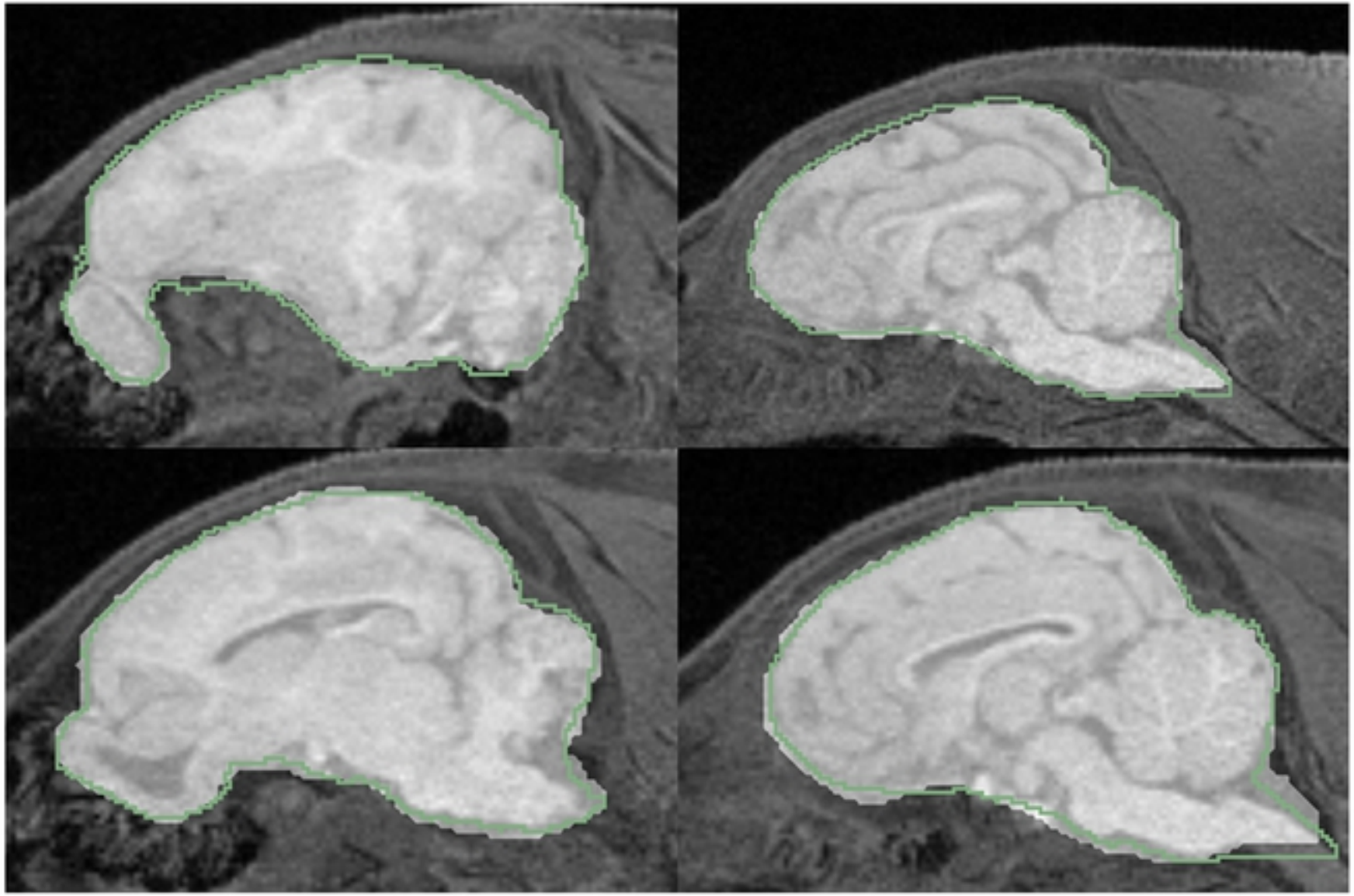

Figure 3

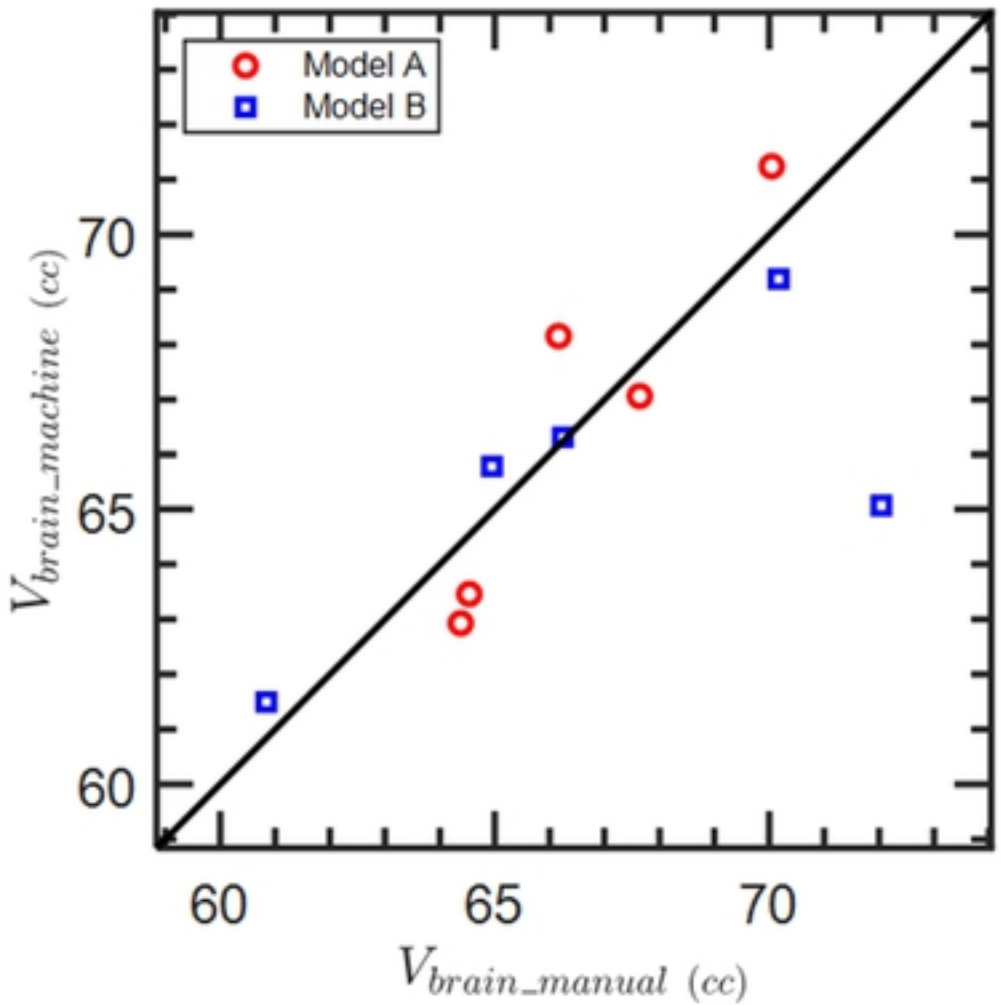
Model A
Model B
70
65
60
60
65
70
$V_{brain_machine}$ (cc)
V_{brain_manual} (cc)

Figure 4

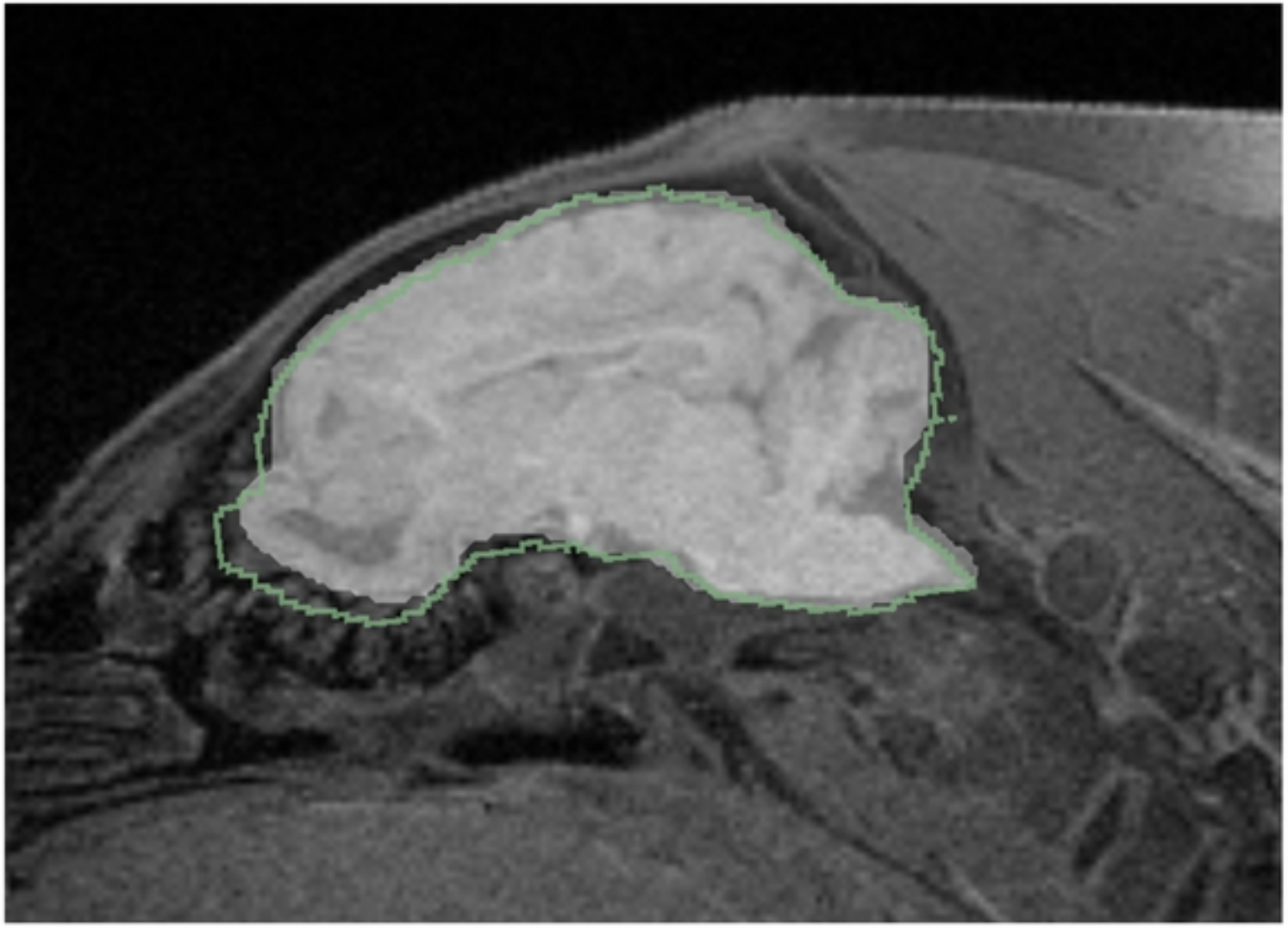

Figure 5