1 **Automated skull stripping in mouse fMRI analysis using 3D U-Net**

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23 **Abstract**

24 Skull stripping is an initial and critical step in the pipeline of mouse fMRI analysis.

25 Manual labeling of the brain usually suffers from intra- and inter-rater variability

26 and is highly time-consuming. Hence, an automatic and efficient skull-stripping

27 method is in high demand for mouse fMRI studies. In this study, we investigated a

28 3D U-Net based method for automatic brain extraction in mouse fMRI studies. Two

29 U-Net models were separately trained on T2-weighted anatomical images and

30 T2*-weighted functional images. The trained models were tested on both interior

31 and exterior datasets. The 3D U-Net models yielded a higher accuracy in brain

32 extraction from both T2-weighted images (Dice > 0.984, Jaccard index > 0.968 and

33 Hausdorff distance < 7.7) and T2*-weighted images (Dice > 0.964, Jaccard index >

34 0.931 and Hausdorff distance < 3.3), compared with the two widely used mouse

35 skull-stripping methods (RATS and SHERM). The resting-state fMRI results using

36 automatic segmentation with the 3D U-Net models are identical to those obtained by

37 manual segmentation for both the seed-based and group independent component

38 analysis. These results demonstrate that the 3D U-Net based method can replace

39 manual brain extraction in mouse fMRI analysis.

40

41 **Keywords:** Skull stripping, Deep learning, 3D U-Net, Mouse, fMRI

42

43 **1 Introduction**

44 Functional magnetic resonance imaging (fMRI) (D'Esposito et al., 1998; Lee et al.,
 45 2013) has been widely employed in neuroscience research. The key advantage of
 46 mouse fMRI (Jonckers et al., 2011; Mechling et al., 2014; Perez-Cervera et al., 2018;
 47 Wehrl et al., 2014) is that it can be combined with neuromodulation techniques (e.g.,
 48 optogenetics) and allows manipulation and visualization of whole-brain neural
 49 activity in health and disease, which builds an important link between pre-clinical
 50 and clinical research (Lake et al., 2020; Lee et al., 2010; Zerbi et al., 2019). In
 51 mouse fMRI research, structural and functional images are commonly acquired with
 52 T2-weighted (T2w) and T2*-weighted (T2*w) scanning, respectively. Generally,
 53 functional T2*w images need to be registered to standard space using two spatial
 54 transformations, which are obtained by registering functional images to anatomical
 55 images and subsequently registering anatomical images to a standard space. To
 56 exclude the influence of non-brain tissues on image registration, it is necessary to
 57 perform skull stripping on both structural and functional images. In the practice of
 58 mouse fMRI analysis, skull stripping is usually performed by manually labeling
 59 each MRI volume slice-by-slice, due to the absence of a reliable automatic
 60 segmentation method. This manual brain extraction is extremely time-consuming, as
 61 a large number of slices need to be processed in the fMRI analysis for each mouse.
 62 In addition, manual segmentation suffers from intra- and inter-rater variability.
 63 Therefore, a fully automatic, rapid, and robust skull-stripping method for both T2w
 64 and T2*w images is highly desirable in mouse fMRI studies.

65 In human research, a number of automatic brain-extraction methods have been
 66 developed and widely used, including Brain Extraction Tool (BET) (Smith, 2002),
 67 Hybrid Watershed Algorithm (HWA) (Segonne et al., 2004), the Brain Extraction
 68 based on nonlocal Segmentation Technique (BEaST) (Eskildsen et al., 2012), and the
 69 Locally Linear Representation-based Classification (LLRC) for brain extraction
 70 (Huang et al., 2014). However, these methods cannot directly be applied for mouse
 71 skull stripping. Compared to human brain MR images, mouse counterparts have
 72 relatively lower tissue contrast and a narrower space between the brain and skull,
 73 which substantially increases the difficulty of mouse brain segmentation. In addition,
 74 the T2*w images used for mouse fMRI may suffer from severe distortion and low
 75 spatial resolution, making the skull stripping of functional images more challenging
 76 than that for structural images.

77 Several methods have been proposed for rodent brain extraction. 3D
 78 Pulse-Coupled Neural Network (PCNN)-based skull stripping (Chou et al., 2011) is
 79 an unsupervised artificial 3D network approach that iteratively groups adjacent pixels
 80 with similar intensity and performs morphological operation to obtain the rodent
 81 brain mask. Rodent Brain Extraction Tool (T. Wood, 2013) is adapted from the
 82 well-known BET (Smith, 2002) method with an appropriate shape for the rodent.
 83 Rapid Automatic Tissue Segmentation (RATS) (Oguz et al., 2014) consists of two
 84 stages: grayscale mathematical morphology and LOGISMOS-based graph
 85 segmentation (Yin et al., 2010), and it incorporates the prior of rodent brain anatomy
 86 in the first stage. SHape descriptor selected Extremal Regions after Morphologically

87 filtering (SHERM) (Liu et al., 2020) is an atlas-based method that relies on the fact
 88 that the shape of the rodent brain is highly consistent across individuals. This
 89 method adopts morphological operations to extract a set of brain mask candidates
 90 that match the shape of the brain template, and then merges them for final skull
 91 stripping. One of the common disadvantages of the above methods is that their
 92 effectiveness was verified only on anatomical images, and cannot be guaranteed on
 93 functional images. Another limitation is that the performance of these methods is
 94 severely affected by the brain shape, texture, signal to noise ratio, and contrast of
 95 images, and hence they need to be optimized for different MRI images. Therefore, it
 96 is necessary to develop an automatic skull-stripping method that is effective and has
 97 a stable performance on varying types of MR images.

98 Deep learning has gained popularity in varying image analysis tasks, such as
 99 organ or lesion segmentation (Guo et al., 2019; Li et al., 2020; Sun et al., 2019), and
 100 disease diagnosis (De Fauw et al., 2018; Suk et al., 2017), owing to its excellent
 101 performance. As for skull stripping, Kleesiek et al. (2016) first proposed a 3D
 102 convolutional neural network (CNN) method for human brain MR images. Roy et al.
 103 (2018) trained a CNN architecture with modified Google Inception (Szegedy et al.,
 104 2015) using multiple atlases for both human and rodent brain MR images. With the
 105 development of deep learning, more advanced architectures of CNN for semantic
 106 segmentation have been proposed. As a popular architecture of deep CNN, U-Net
 107 has proved to be very effective in the task of semantic segmentation even with a
 108 limited amount of annotated data (Ronneberger et al., 2015). Some U-Net based

109 methods for rodent skull stripping have been proposed. Thai et al. (2019) utilized 2D
 110 U-Net for mouse skull stripping on diffusion weighted images. Hsu et al. (2020)
 111 trained a 2D U-Net based model using both anatomical and functional brain images
 112 for mouse and rat skull stripping. De Feo et al. (2021) proposed a multi-task U-Net
 113 to accomplish both skull stripping and brain region segmentation simultaneously on
 114 mouse anatomical images of the mouse brain. These methods were only evaluated
 115 using segmentation accuracy with reference to the ground truth mask. However, the
 116 effect of skull-stripping algorithm on the final fMRI results remains unexplored, and
 117 whether automatic skull stripping can replace the manual approach in mouse fMRI
 118 analysis remains an open question.

119 Here, we investigated the feasibility of using 3D U-Net to extract the mouse brain
 120 from T2w anatomical and T2* functional images for the fMRI analysis. We
 121 separately trained the U-Net models on anatomical T2w and functional T2*w
 122 images, considering that anatomical and functional images are acquired with
 123 different sequences and have different contrast, resolution, and artifacts. The
 124 performance of trained 3D U-Net models is first quantitatively evaluated using
 125 conventional accuracy metrics. In addition, we compared the fMRI results separately
 126 obtained using the manual and automatic segmentation masks.

127

128 **2 Materials and Methods**

129 **2.1 Datasets**

130 This study includes two different in-house datasets. Both of these two datasets

131 were collected in the fMRI study and were reanalyzed for the purpose of the present
132 study. All animal experiments were approved by local Institutional Animal Care and
133 Use Committee.

134 The first dataset (D1) was acquired from 84 adult male C57BL/6 mice (25–30 g)
135 on a Bruker 7.0T MRI scanner using cryogenic RF surface coils (Bruker, Germany).
136 Both the anatomical data (T2w) and resting-state fMRI data (T2*w) were acquired
137 for each mouse. The T2w images were acquired using a fast spin echo (TurboRARE)
138 sequence: field of view (FOV) = $16 \times 16 \text{ mm}^2$, matrix = 256×256 , in-plane
139 resolution = $0.0625 \times 0.0625 \text{ mm}^2$, slice number = 16, slice thickness = 0.5 mm,
140 RARE factor = 8, TR/TE = 2500 ms/35 ms, number of averages = 2. The
141 resting-state fMRI images were then acquired using a single-shot
142 gradient-echo-planar-imaging sequence with 360 repetitions: FOV = $16 \times 16 \text{ mm}^2$,
143 matrix size = 64×64 , in-plane resolution = $0.25 \times 0.25 \text{ mm}^2$, slice number = 16,
144 slice thickness = 0.4 mm, flip angle = 54.7° , TR/TE = 750 ms/15 ms.

145 The second dataset (D2) was obtained from the previous task-state fMRI
146 research (Chen et al., 2020). A total of 27 adult male C57BL/6 mice (18–30 g) were
147 used in this study (Part 1: 13 for auditory stimulation; Part 2: 14 for somatosensory
148 stimulation). The anatomical T2w images and two sets of functional T2*w images
149 were acquired for each mouse with the Bruker 9.4T scanner. The T2w images were
150 acquired using a TurboRARE sequence: FOV = $16 \times 16 \text{ mm}^2$, matrix = $256 \times$
151 256 , in-plane resolution = $0.0625 \times 0.0625 \text{ mm}^2$, slice number = 32, slice
152 thickness = 0.4 mm, RAREfactor = 8, TR/TE = 3200 ms/33 ms. Two sets of

functional T2*w data, acquired using a single-shot echo planar imaging (EPI) sequence, consist of a high spatial resolution one (EPI01) and a high temporal resolution one (EPI02). Parameters for EPI01 were: FOV = $15 \times 10.05 \text{ mm}^2$, matrix size = 100×67 , in-plane resolution = $0.15 \times 0.15 \text{ mm}^2$, slice number = 15, slice thickness = 0.4 mm, flip angle = 60° , TR/TE = 1500 ms /15 ms, repetitions = 256. EPI02 images were acquired with the following parameters: FOV = $15 \times 12 \text{ mm}^2$, matrix size 75×60 , in-plane resolution = $0.2 \times 0.2 \text{ mm}^2$, slice number = 10, slice thickness = 0.4 mm, flip angle = 35° , TR/TE = 350 ms/15 ms, repetitions = 1100.

2.2 U-Net

The U-Net architecture is shown in Figure 1. It consists of one encoding and one decoding path with a skip connection. The skip connection, which connects the corresponding downsampling and upsampling stages, allows the model to integrate multi-scale information and better propagate the gradients for improved performance. Notably, the anatomical and functional images were usually acquired slice-by-slice with 2D sequence to span the whole brain in the fMRI study, resulting in an intra-slice spatial resolution that is significantly higher than inter-slice spatial resolution. In such case, the conventional approach is to take the individual 2D slice as input and predict the corresponding brain mask with 2D U-Net. However, the 2D model ignores the inter-slice information. To capture more spatial feature information while preserve intra-slice information, we took the multi-slice

Figure 1 – Architecture of the 3D U-Net for skull stripping. Each blue box indicates a multi-channel feature map, and the number of channels is denoted on top of the box. Every white box indicates the copied feature map. Color-coded arrows denote the different operations.

175

176 2D images as an anisotropic 3D volume and used 3D U-Net model for training by
 177 only maxpooling and upsampling within the slice.

178 Every stage in both the encoding and decoding path is composed of two
 179 repeated $3 \times 3 \times 3$ convolutions, each of which is followed by a rectified linear
 180 unit (ReLU), and a 2×2 slice max-pooling layer (in the encoding path) or a 2×2
 181 slice deconvolutional layer (in the decoding path). The number of downsampling
 182 and upsampling is set to three, and the channel number of first stage is set to 32,
 183 being doubled at each downsampling step and then halved at each upsampling step.
 184 The output layer following the last decoding stage is a $1 \times 1 \times 1$ single channel
 185 convolutional layer with a sigmoid activation function, which transforms the feature
 186 representation to one segmentation map.

187 Considering that the appearances of T2w images were significantly different
188 from those of T2*w images, using the data from both modalities to train a network
189 may degrade the segmentation performance. In this study, two models were trained
190 separately with anatomical T2w images and functional T2*w images based on the
191 above 3D U-Net architecture. As functional T2*w data include multiple repetitions in
192 each section, only the first repetition was used in our study. A total of 74 mice were
193 randomly selected from the first dataset for training, and the remaining 10 mice were
194 used for testing. All of the second dataset were also used as test data to verify the
195 effectiveness of our model across different data sources. In the training phase, 80% of
196 training data from 74 mice were selected randomly to train the model, and the
197 remaining 20% was used to validate the model to avoid over-fitting. Furthermore,
198 data augmentation like flipping and rotating around three axes was also used to
199 increase the diversity of the training dataset to improve the generalization of the
200 models.

201 The 3D U-Net models was implemented using TensorFlow1.12.0 (Abadi et al.,
202 2016) and trained on a Nvidia Titan X GPU (12GB). The convolution parameters
203 were randomly initialized from a normal distribution with mean value of zero and a
204 standard deviation of 0.001. Adam was used to optimize the training network
205 (Kingma and Ba, 2014) with mini-batch size of two, and batch normalization (Ioffe
206 and Szegedy, 2015) was adopted to accelerate the network training. The maximal
207 number of training epochs was set to 100. The learning rate started from 10^{-4} and
208 decayed by a factor of 0.99 every epoch. The loss function used to train the models

209 was focal loss (Lin et al., 2020), which is designed to address class imbalance and can
210 focus training on a sparse set of hard examples with large errors.

211

212 **2.3 Comparison of methods**

213 3D U-Net models were compared to two widely used methods for mouse brain
214 extraction: RATS and SHERM. The parameters of both methods were carefully
215 tuned to achieve the best performance for each dataset. For RATS (Oguz et al.,
216 2014), the brain volume was set to 380 mm³ for both D1 and D2. In D1, the intensity
217 threshold was set to 2.1 times of average intensity of each image for the T2w modality
218 and 1.2 times for the T2*w modality. In D2, the threshold value was set to 1.3 times
219 for the T2w and 0.6 times for the T2*w. For SHERM (Liu et al., 2020), the range of
220 brain volume was set to 300–550 mm³. Except for the above parameters, all others
221 were set to the default values for RATS and SHERM.

222

223 **2.4 Data pre- and post-processing**

224 All images were preprocessed before being fed into the models as follows. First,
225 the N4bias field correction (Tustison et al., 2010) was applied to correct the intensity
226 inhomogeneity for all images with the Python SimpleITK (Yaniv et al., 2018).
227 Second, we resampled all data to a resolution of 0.0625 × 0.0625 mm² for the
228 anatomical T2w images and 0.25 × 0.25 mm² for the functional T2*w images
229 within the slice, leaving the inter-slice resolution unchanged. Then, all images were
230 zero-padded to a size of 256 × 256 for the anatomical images and 64 × 64 for the

functional images within slices. The padding was not performed in the inter-slice direction when the slice number was larger than 20, otherwise the slice number was padded to 20 with edge values of the slice. Subsequently, the histogram of each image was matched to a target histogram that was the average of all histograms of the training dataset. Finally, each anisotropic volume data was normalized in the range [0, 1].

After obtaining the binary mask from the network's probability output, the only post-processing step was to identify the largest connected component and discard all the others (disconnected ones) for the final brain mask.

2.5 Evaluation methods

Two types of assessment methods were used to evaluate the effectiveness of the 3D U-Net models. The first type measured the overlap of the predicted segmentation mask ($M_{predicted}$) generated by each skull-stripping algorithm and the manual segmentation mask (M_{manual}). The quantify metrics include the Dice coefficient, the Jaccard index, and the Hausdorff distance.

The Dice coefficient is defined as twice the size of the intersection of the two masks divided by the sum of their sizes:

$$\text{Dice} = \frac{2|M_{manual} \cap M_{predicted}|}{|M_{manual}| + |M_{predicted}|} \quad (1)$$

The Jaccard index is defined as the size of the intersection of the two masks divided

253 by the size of their union:

$$254 \quad \text{Jaccard} = \frac{|M_{\text{manual}} \cap M_{\text{predicted}}|}{|M_{\text{manual}} \cup M_{\text{predicted}}|} \quad (2)$$

255

256 The Hausdorff distance (Huttenlocher et al., 1993) between two finite point sets is

257 defined as:

$$258 \quad \text{Hausdorff} = \max \left\{ \sup_{x \in X} \inf_{y \in Y} \|x - y\|, \sup_{y \in Y} \inf_{x \in X} \|x - y\| \right\}, \quad (3)$$

259 where X and Y denote the boundaries of the predicted segmentation mask and the

260 manual segmentation mask, respectively. To exclude possible outliers, the Hausdorff

261 distance is redefined as the 95th percentile distance instead of the maximum in our

262 study.

263 The second assessment involves evaluating the impact of automatic skull stripping

264 with 3D U-Net models on the final result of the fMRI analysis. The fMRI data were

265 preprocessed with the common pipeline after skull stripping and then analyzed with

266 seed-based analysis (Chan et al., 2017; Wang et al., 2019) and group independent

267 component analysis (group ICA) (Mechling et al., 2014; Zerbi et al., 2015), which are

268 two widely used methodologies in fMRI studies. Seed-based analysis requires

269 creating a seed region first and then generates a seed-to-brain connectivity map by

270 calculating the Pearson's correlation coefficient (CC) between the BOLD time course

271 of the seed and those of all other voxels in the brain. Group ICA is a data-driven

272 method for the blind source separation of fMRI data, which is implemented in the

273 group ICA of fMRI toolbox (GIFT) (Rachakonda et al., 2007). The Pearson's

274 correlation coefficient was calculated between the time courses of each pair of

275 components extracted from the group ICA.

276

277 **3 Results**

278 Figure 2 illustrates the segmentation results of RATS, SHERM, and the 3D U-Net
 279 model on the T2w images from one mouse in D1 (figure 2a) and from two mice of
 280 auditory (**b**) and somatosensory (**c**) stimulation in D2. As shown in Figure 2a, all three
 281 methods successfully extracted most brain tissue. By comparison, the segmentation
 282 contours of the 3D U-Net model and SHERM were smoother and closer to the ground
 283 truth than those of RATS. However, the brain mask predicted by SHERM misaligned
 284 with the ground truth at the sharp-angled corner, while the 3D U-Net model still
 285 performed well in these locations. As shown in Figure 2b and 2c, the segmentation
 286 performance on T2w images of D2 was degraded for RATS and SHERM, compared
 287 to that of D1. The performance degradation might be attributed to T2w images of D2
 288 having lower SNR and image contrast than those of D1. Specifically, the RATS
 289 produced a brain mask with a rough boundary, while the SHERM evidently
 290 overestimated the entire brain volume on D2. However, the 3D U-Net model still
 291 performed well on both D2 and D1.

292 Figure 3 shows the segmentation results for three representative slices of RATS,
 293 SHERM, and the 3D U-Net model on T2*w images from one mouse in D1 (Figure 3a)
 294 and from four mice in D2 with high spatial resolution (EPI01) (Figure 3b_1, 3c_1)
 295 and high temporal resolution (EPI02) (Figure 3b_2, 3c_2). The SHERM
 296 overestimated the brain volume, especially for the D2. The RATS and 3D U-Net

297 model performed better than SHERM in both D1 and D2. However, the brain
298 boundaries predicted by the RATS were misaligned with the ground-truth boundaries
299 in the areas with severe distortions or signal losses (white arrows), while the 3D
300 U-Net model still had an accurate alignment on these locations.

Figure 2 – Example segmentation comparison for T2w images from one mouse in D1 (a) and from two mice with auditory (b) and somatosensory (c) stimulation in D2. Red lines show the contours of ground truth; yellow lines show automatically computed brain masks by RATS, SHERM, and the 3D U-Net model. White arrows point to the rough boundary, where the 3D U-Net model performed better than RATS and SHERM.

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Figure 3 – Example segmentations comparison for T2*w images from one mouse in D1 (a) and from two mice with auditory stimulation (b) and two mice with somatosensory stimulation (c) in D2; **b_1**, **c_1** represent EPI01, and **b_2**, **c_2** represent EPI02. Red lines show the contours of ground truth; yellow lines show automatically computed brain masks by RATS, SHERM, and the 3D U-Net model. White arrows point to the rough boundary, where 3D U-Net models performed better than RATS and SHERM.

304 Tables 1 and Table 2 show the quantitative assessment of RATS, SHERM, and
 305 3D U-Net models for T2w images and T2*w images, respectively. The 3D U-Net
 306 models yielded highest mean values of the Dice and Jaccard index, and lowest mean
 307 values of the Hausdorff distance in both T2w and T2*w images. In addition, the
 308 standard deviations of all three metrics were lowest for the 3D U-Net models in most
 309 cases, except for the Hausdorff distance on T2w images of D2_PART2, and the Dice
 310 and Jaccard index on T2*w images of both D1 and D2_PART2_EPI01, which were
 311 slightly higher than those of RATS. The above quantitative results indicate that the 3D
 312 U-Net models exhibit a high accuracy and stability.

313 Figure 4 compares the results of seed-based analysis from one mouse in the test
 314 data of D1 with automatic skull stripping by the 3D U-Net models and manual brain
 315 extraction. The seeds (2×2 voxels) were positioned in the dorsal striatum (dStr),
 316 somatosensory barrel field cortex (S1BF), secondary somatosensory cortex (S2), and
 317 ventral striatum (vStr). The CC maps with our model were similar to those with
 318 manual brain extraction. We also plotted scatter plots, where the horizontal axis
 319 represents the values of CC maps with predicted masks, and the vertical axis
 320 represents the values of CC maps with the manual mask. Each point represents a pair
 321 of the two values at the same pixel location. All points were concentrated on the
 322 diagonal with $R^2 = 1$. The above results indicate that the CC maps with automatic
 323 skull stripping by 3D U-Net models were identical to those with manual brain
 324 extraction.

325 Figure 5 compared the four components of group ICA analysis with automatic

326

327 Table 1 – Mean and standard deviation of Dice, Jaccard index, and Hausdorff distance
328 evaluating the RATS, SHERM, and 3D U-Net model for T2w images in different
329 datasets. Bold values indicate the best results.

Dataset	Method	Dice	Jaccard index	Hausdorff distance
D1	RATs	0.9377±0.0036	0.8826±0.0064	8.97±0.99
	Sherm	0.9767±0.0028	0.9545±0.0054	6.02±1.32
	Proposed	0.9898±0.0013	0.9800±0.0025	3.53±0.86
D2_PART1 (auditory)	RATs	0.9404±0.0038	0.8875±0.0068	8.77±1.61
	Sherm	0.9686±0.0079	0.9392±0.0147	20.28±8.55
	Proposed	0.9842±0.0014	0.9690±0.0027	6.04±1.08
D2_PART2 (somatosensory)	RATs	0.9442±0.0049	0.8943±0.0087	9.69±1.61
	Sherm	0.9735±0.0048	0.9484±0.0091	16.17±6.04
	Proposed	0.9845±0.0023	0.9696±0.0044	6.95±1.96

330

331 skull stripping by the 3D U-Net models and manual brain extraction. The presented
332 four components in Figure 5 correspond to the same regions used in the
333 seed-based analysis (S1BF, S2, dStr, vStr). The component maps with 3D U-Net
334 models for skull stripping were visually similar to those with manual brain extraction.
335 The points in the scatter plots were concentrated on the diagonal with $R^2 = 1$. These
336 results indicate that the component maps of group ICA with automatic skull stripping
337 by 3D U-Net models are highly consistent with those with manual brain extraction.
338 Group ICA analysis with 3D U-Net models generated the same 32 independent
339 components as those with manual brain extraction. The regions corresponding to each
340 component are shown in Supplementary Table S1.

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Table 2 – Mean and standard deviation of Dice, Jaccard index, and Hausdorff distance evaluating the RATS, SHERM, and 3D U-Net model for T2*w images in different datasets. Bold values indicate the best results.

Dataset	Method	Dice	Jaccard index	Hausdorff distance
D1	RATs	0.9467± 0.0028	0.8989± 0.0051	2.98±0.48
	SHERM	0.9070±0.0125	0.8301±0.0211	5.17±1.82
	Proposed	0.9644 ±0.0044	0.9313 ±0.0083	2.95±0.56
D2_PART1_EPI01 (auditory)	RATs	0.9504± 0.0031	0.9057± 0.0056	4.49±0.78
	SHERM	0.8306±0.1108	0.7260±0.1665	17.18±9.04
	Proposed	0.9644 ±0.0044	0.9313 ±0.0083	2.95±0.56
D2_PART1_EPI02 (auditory)	RATs	0.9453±0.0206	0.8969±0.0353	3.56±1.54
	SHERM	0.8887±0.0388	0.8018±0.0609	9.90±3.53
	Proposed	0.9663 ± 0.0078	0.9350 ± 0.0144	2.57±0.94
D2_PART2_EPI01 (somatosensory)	RATs	0.9484±0.0064	0.9019±0.0116	5.52±1.06
	SHERM	0.8939±0.1040	0.8226±0.1538	12.21±7.63
	Proposed	0.9694 ± 0.0037	0.9406 ± 0.0070	3.30±0.59
D2_PART2_EPI02	RATs	0.9516±0.0076	0.9077±0.0139	3.74±0.75
	SHERM	0.9284±0.0241	0.8674±0.0411	6.70±1.87
	Proposed	0.9650 ± 0.0068	0.9326 ± 0.0126	2.83±0.75

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348 Figure 6 shows the functional network connectivity (FNC) correlations for one
349 mouse and the average FNC correlation across 10 mice between each pair of 32
350 regions extracted from group ICA analysis. Each correlation value in FNC matrixes

Figure 4 – Exemplary results of seed-based analysis for one mouse in the test data of D1. The selected seed regions were S1BF, S2, dStr, and vStr. The left column illustrates the CC maps with manual brain extraction and automatic skull stripping by the 3D U-Net models for each seed region. The corresponding right column illustrates the scatters, where each point represents a pair of the two values from different CC maps with manual brain extraction and automatic skull stripping by the 3D U-Net models, at the same pixel location.

Figure 5 – Example results of ICA analysis for test data of D1. Four selected components extracted by group ICA matching the four seed regions in seed-based analysis are shown in each row. The left column illustrates the component maps with manual brain extraction and automatic skull stripping by 3D U-Net models. The right column illustrates the scatters, where each point represents a pair of the two values from different component maps with manual brain extraction and automatic skull stripping by the 3D U-Net models, respectively, at the same pixel location.

353 with automatic skull stripping by 3D U-Net models was identical to that obtained with
354 manual brain extraction. The points in the scatter plots are concentrated on the
355 diagonal with $R^2 = 1$, which indicates that the use of the 3D U-Net models for skull
356 stripping in the fMRI analysis pipeline does not affect the final results of group ICA
357 based FC analysis. The FNC correlations of the other nine mice are shown in
358 Supplementary Figure S1.

359 **4 Discussion**

360 To the best of our knowledge, this is the first study investigating the feasibility of
361 3D U-Net for mouse skull stripping from brain functional (T2*w) images and the
362 impact of automatic skull stripping on the final fMRI analysis. Results indicate that
363 the 3D U-Net model performed well on both anatomical (T2w) and functional (T2*w)
364 images and achieved state-of-the-art performance in mouse brain extraction. The
365 fMRI results with automatic brain extraction using 3D U-Net models are nearly
366 identical with those with manual brain extraction. Thus, the manual brain extraction in
367 the fMRI pre-processing pipeline can be replaced by the proposed automatic
368 skull-stripping method.

369 The 3D U-Net models were tested on not only interior but also exterior datasets.
370 Notably, the exterior datasets were acquired with different acquisition parameters on a
371 scanner with different field strength from another MRI center. The results show that
372 3D U-Net models had high segmentation accuracy that is comparable between interior
373 and exterior datasets. This demonstrates that the developed method has high reliability
374

Figure 6 –Functional connectivity between the independent components extracted from group ICA analysis for the test data of D1. The left column illustrates the FNC matrixes from one mouse, and the right column illustrates the average FNC matrixes across 10 mice. Scatter plots are shown in the bottom row, where each point represents the difference between two values from two FNC matrixes with manual brain extraction and automatic skull stripping by the 3D U-Net models, at the same pixel location.

and excellent generalization ability. The 3D U-Net models also outperformed two widely used brain extractions for rodent (SHERM and RATS). SHERM has the second best performance in T2w images (Figure 2), while it has the worst performance in T2*w images (Figure 3). The reason for this is that the poor quality of T2*w images makes it difficult to match the shape of the brain template. Although RATS has stable performance across different datasets and modalities, its segmentation accuracy (Dice < 0.945 in T2w images and Dice < 0.952 in T2*w images) is consistently lower than in our method (Dice > 0.984 in T2w images and Dice > 0.964 in T2*w images).

There are several related reports on using U-Net for mouse skull stripping (De Feo et al., 2021; Hsu et al., 2020; Thai et al., 2019). Compared with the model adopted by Hsu et al. (2020), the segmentation accuracy of our models is relatively higher in both T2w and T2*w images. The first reason is that we used 3D U-Net for mouse skull stripping, while Hsu et al. used 2D U-Net. The second reason is that we trained the U-Net models separately for T2- and T2*w images. The performance of our 3D U-Net model is comparable to that of the 3D model adopted by De Feo et al. (2021) on T2w anatomical images. We also applied the 3D U-Net for brain extraction from T2*w functional images. The multi-task U-Net developed by De Feo et al. can hardly be applied to functional images, because it is difficult to delineate different brain regions in functional images due to their low spatial resolution, contrast, signal-to-noise ratio, and severe distortion.

It is essential to guarantee that automatic skull-stripping method does not alter

398 fMRI analysis results. Thus, we not only evaluated the segmentation accuracy, but
399 also investigated the effect of automatic segmentation on fMRI analysis results. The
400 fMRI analysis results with automatic skull stripping by 3D U-Net models are identical
401 to those with manual skull stripping. This finding demonstrates that the 3D U-Net
402 based method can replace manual skull stripping and facilitate the establishment of
403 the automated fMRI analysis pipeline for the mouse model.

404 With respect to the computational cost, the 3D U-Net based method proves to be
405 time efficient. The computation time of the 3D U-Net method was approximately 3 s
406 for a T2w volume data with a size of $256 \times 256 \times 20$, and 0.5 s for T2*w volume
407 data with a size of $64 \times 64 \times 20$. In comparison, the computation time of SHERM
408 is 780 s and 3 s for T2w and T2*w images, respectively; the computation time of
409 RATS is 10 s for T2w images and 3 s for T2*w images. All test procedures were run
410 on a server with a Linux 4.15.0 system, an Intel(R) Xeon(R) E5-2667 8-core CPU,
411 and 256 GB RAM.

412 There are two limitations in our current work. First, the segmentation accuracy of
413 the developed 3D U-Net model on functional images is still relatively lower than that
414 on anatomical images, because of the poor image quality of the functional images.
415 Utilizing the cross-modality information between anatomical and functional images
416 may further improve the accuracy of skull stripping on functional images. Second, the
417 developed 3D U-Net model was only trained and validated on adult C57BL/6 mice,
418 and cannot be directly applied to brain MR images from different mouse types and
419 ages. To address this problem, the model need to be retrained by including more

manually labeled data from mice with varying types and ages. Labeling data is time-consuming and labor-intensive, and another potential approach to reduce the amount of labeled data is to utilize transfer learning (Long et al., 2015; Yu et al., 2019; Zhu et al., 2021).

5 Conclusion

We investigated an automatic skull-stripping method based on 3D U-Net for mouse fMRI analysis. The 3D U-Net based method achieves state-of-the-art performance on both T2w and T2*w images in terms of the segmentation accuracy. Identical results between mouse fMRI analysis using manual and automatic skull stripping demonstrates that the 3D U-Net model has a great potential to replace manual labeling in the mouse fMRI analysis pipeline. Hence, skull stripping by the 3D U-Net model will facilitate the establishment of an automatic pipeline of mouse fMRI data processing.

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