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A PRELIMINARY VOLUMETRIC MRI STUDY OF AMYGDALA AND HIPPOCAMPAL SUBFIELDS IN AUTISM DURING INFANCY

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Abstract

Currently, autism spectrum disorder (ASD) is mainly diagnosed by the observation of core behavioral symptoms. Consequently, the window of opportunity for effective intervention may have passed, when the disorder is detected until 3 years of age. Thus, it is of great importance to identify imaging-based biomarkers for early diagnosis of ASD. Previous findings indicate that an abnormal pattern of the amygdala and hippocampal development in autism persists through childhood and adolescence. However, due to the low tissue contrast and small structural size of amygdala and hippocampal subfields, our knowledge on their growth in autistics in early stage still remains very limited. In this paper, for the first time, we propose a volume-based analysis of the amygdala and hippocampal subfields of the infant subjects with risk of ASD at around 24 months of age. Specifically, to address the challenge of low tissue contrast, we propose a novel deep-learning approach, i.e., dilated-dense U-Net, to automatically segment the amygdala and hippocampal subfields. Experimental results on National Database for Autism Research (NDAR) show the advantages of our proposed method in terms of segmentation accuracy. Our volume-based analysis shows the overgrowths of amygdala and CA1–3 of hippocampus, which may link to the emergence of autism spectrum disorder.

Keywords

early autism diagnosis; segmentation; hippocampal subfields; amygdala

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1. INTRODUCTION

Autism, or autism spectrum disorder (ASD), refers to a range of conditions characterized by challenges with social skills, repetitive behaviors, speech and nonverbal communication, as well as by unique strengths and differences. Globally, autism is estimated to affect 24.8 million people as of 2015 [1]. The diagnosis of ASD is mainly based on behaviors. Studies demonstrate that behavioral signs could begin to emerge as early as 6 to 12 months of age. However, most professionals who specialize in diagnosing the disorder won't attempt to make a definite diagnosis until 3 or 4 years of age. As a result, the window of opportunity for effective intervention may have passed when the disorder is detected. Thus, it is of great importance to detect ASD earlier in life for better intervention [2].

Amygdala and hippocampus have been implicated in deficits associated with ASD, including social cognition, perception of eye-gaze direction, and emotion [5]. For example, some studies have reported decreased amygdala volume [3–4] during adolescence. Whereas others have found increased amygdala and hippocampal volumes [5–7] from childhood to young adult. However, most of the autistic subjects involved in previous studies focused on childhood and adolescence. Our knowledge on the volumetric growth of autistics in early stage still remains very limited. Moreover, studies on hippocampal subfields, i.e., subiculum, cornu ammonis (CA) 1–3, and dentate gyrus (DG) [8], are rare at the early stage. In fact, each subfield has different function, e.g., CA3 and dentate gyrus (CA3&DG) are involved in memory encoding and early retrieval and CA1 is involved in late retrieval, consolidation and recognition [9]. Investigating volumes of amygdala and hippocampal subfields with autism at an early age may therefore reveal crucial insights in the neurobiology of autism.

To characterize amygdala and hippocampal subfields at 24-month old of age, it is critically important to accurately segment them from MR images. Manual segmentation is often treated as gold standard, but it is very time-consuming and tedious, along with large inter- and intra-observer variability. In recent years, deep neural networks have been widely applied in medical image segmentation. Fully convolutional networks (FCNs) [10], as a natural extension of convolutional neural networks (CNNs), were developed for semantic segmentation of natural images and have been rapidly applied in biomedical images due to their powerful end-to-end training. The U-Net [11] further extends the FCNs for volumetric segmentation by using a skip connection to capture both the local and contextual information. To date, many network architectures further incorporate the residual connection [12] or dense connection [13] to these previous networks to get an efficient improved flow of information and gradients throughout the network [14–15]. A new convolutional network module which is specifically designed for dense prediction was proposed in [17]. The module uses dilated convolutions to systematically aggregate multiscale contextual information without losing resolution.

Inspired by [16–17], in this paper, we propose a novel Dilated-Dense U-Net (DDUNET) for accurate segmentation of amygdala and hippocampal subfields from around 24-month-old infant brain MRI. Based on the segmentation results, we further perform a volume-based analysis to identify group difference between ASD and normal controls (NC).

2. METHOD

Dataset and preprocessing:

A total of 271 subjects gathered from National Database for Autism Research (NDAR) [18] were used in the study. More specifically, the dataset consists of 211 normal controls, 30 mild condition autism spectrum subjects, and 30 autistic subjects. In the experiments, we regard the last two types as one group. Clinical diagnostic and gender information are listed in Table I. All images were acquired at around 24 months of age on a Siemens 3T scanner, while subjects were naturally sleeping and fitted with ear protection, with their heads secured in a vacuum-fixation device. T1-weighted MR images were acquired with 160 sagittal slices using parameters: TR/TE=2400/3.16ms and voxel resolution= $1\times1\times1\text{mm}^3$. Then, in-house tools were used to perform skull stripping, intensity inhomogeneity correction, and histogram matching for MR images. Fifteen subjects were manually labeled by an experienced neuroscientist (Dr. Meng-Hsiang Chen, MD, Ph.D.): 3 subjects (0F/3M) are from the ASD group while 12 subjects (3F/9M) are from the NC group.

Dilated-Dense U-Net:

In this study, a novel architecture, Dilated-Dense U-Net (DDUNET), was proposed to segment the amygdala and hippocampal subfields. Inspired by [16–17], the proposed network is a fully convolutional neural network, taking advantage of the U-Net skip connections, dense block and dilated convolutions. The U-Net skip connections allow capturing both local and contextual information, while the dense blocks allowed a better flow of the gradient information. Dilated convolutions support exponential expansion of the receptive field without loss of resolution or coverage, which can effectively alleviate the issue related with small sizes of amygdala and hippocampal subfields.

The proposed network architecture is shown in Fig. 1. It consists of a contracting path and an expansive path, with skip-connection going through 7 dense blocks. Each path consists of one standard dense block and two dilated dense blocks. After the first standard dense block in the contracting path, a max-pooling layer of size $2\times2\times2$ is used to reduce the dimensionality and exploit the contextual information. Each dense block consists of three BN-ReLU-Conv-Dropout operations, in which each Conv includes $f=16$ $3\times3\times3$ kernels and the dropout rate is 0.1. It should be noted that two dilated dense blocks with dilation rates $d=2$ were used in the expansive path to expand receptive fields. Then a dense block with a dilation rate $d=2$ is used to transfer the features from the contracting path to the expansive path.

Network Implementation.

We randomly extracted 3D patches from training images, with at least one voxel belonging to amygdala or hippocampal subfields. The patch size was optimized as $16\times64\times16$. The loss function was based on cross-entropy. We used SGD optimization strategy. The learning rate was 0.005 and decreased by 0.1 after each epoch. Training and testing were performed on an NVIDIA Titan X GPU. Basically, training the proposed network took around 72 hours, and testing on a typical image took 60 seconds.

3. EXPERIMENT RESULTS AND DISCUSSION

In this section, we present our segmentation results of amygdala and hippocampal subfields using the proposed Dilated-Dense U-Net, by comparisons with 2 state-of-the-art networks. Based on the segmentation results, we further measured volumetric differences of amygdala and hippocampal subfields between ASD and NC groups.

3.1. Segmentation results and performance

Five-fold cross validation was used in the experiment. In each fold, we selected 12 subjects for training, and the rest 3 subjects were used for validation and testing. Fig. 2 shows the 2D and 3D views of segmentation results for a randomly selected subject, obtained by different comparison methods including DRUNET [15], SegNet [19] and U-Net [11]. It can be seen that the proposed method derives a more consistent result with the manual segmentation result. Table II reports the Dice coefficients (mean $\pm$ std) of the segmentation results obtained by different methods. Our proposed method achieves a better performance in terms of Dice ratio than other comparison methods. We then apply our trained model on other 256 infants and further perform ROI-based volumetric measurements.

3.2. Volumetric measurements and discussion

Volumetric measurements for all participants are given in Fig. 3. Linear regression scatter plots show the relationship between the volume of each ROI and age. Compared with the NC group, the ASD group shows significant enlargement on amygdala (p -value < 0.01) and hippocampus (p -value < 0.05) in each hemisphere and the whole brain. The degree of amygdala enlargement at early age was associated with the severity of social and communication and emotional perception [20–21]. Especially, for hippocampus, the most significantly different region between ASD and NC groups is CA1–3, with 4.7% and 3.2% enlargements in the left and right CA1–3, respectively. The enlargement of CA1–3 may represent up-regulation, strengthen emotion of fear to communicate with surrounding or others. These findings suggest that there are developmental abnormalities in amygdala and hippocampus (especially for CA1–3) in early age of ASD, which is also confirmed by previous reports on older children and young adults. For example, it was found in [4] that the right amygdala and left hippocampus were significantly enlarged in the ASD group, compared with the NC group.

To eliminate the possible age disturbance, we only focus on the subjects at 24 months of age, with results shown in Fig. 4. For all subjects, there is significant difference in amygdala (both left and right hemispheres, p -value < 0.01), CA1–3 (left hemisphere, p -value < 0.01 ; right hemisphere, p -value < 0.05), and subiculum (left hemisphere, p -value < 0.05) between ASD and NC groups. We also found that males in the ASD group showed more significant abnormality of the amygdala and hippocampal volumes at 24 months of age than males in the NC group. However, no significance was found for female subjects between ASD and NC groups, likely caused by the small sample size (with the number of female ASD = 10).

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These findings provide evidence that the amygdala and CA1–3 in hippocampus are enlarged at the early age with autism, and that the overgrowth may even begin before 2 years of age. This urges us to further explore the growth trajectory of amygdala and hippocampal subfields at earlier stage, e.g., 12 months or 6 months of age.

4. CONCLUSION

In this paper, for the first time, we proposed a volume-based analysis on the amygdala and hippocampal subfields of the infant subjects with risk of ASD at around 24 months of age. First, we proposed a novel Dilated-Dense U-Net to parcellate the amygdala and hippocampal subfields of infant brain MR images. Then, based on accurate segmentations, we performed volume-based analysis of amygdala and hippocampal subfields and found the overgrowth of amygdala and CA1–3 in hippocampus, which may provide crucial insights into the neurobiology of autism and help for possible early diagnosis.

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Fig. 1.

DDUNET consists of two paths: A contracting path to capture the contextual information and an expansive path to capture the local information. Each path consists of three dense blocks: a standard dense block, and two dilated dense blocks.

Fig. 2. Segmentation of amygdala and hippocampal subfields of a typical testing subject, obtained by DRUNET [15], SegNet [19], U-Net [11], the proposed DDUNET, and manual expert, respectively.

Fig. 3.

Linear regression of scatter plots show that the amygdala and CA1–3 volumes are significantly different between the ASD and NC groups in both left and right hemispheres as well as the whole brain. Significance of the p -value of each ROI is shown at the corner of each plot figure, with ** indicating p -value < 0.01 and * indicating p -value < 0.05.

Fig. 4. Significance of the p -value of each ROI, between ASD and NC groups at 24 months of age, is shown, with ** denoting p -value < 0.01 and * denoting p -value < 0.05. The corresponding ROIs in the figure are shown in the table below. The length of box is the interquartile range computed from Tukey’s hinges. The line inside the box is median, and whiskers represent the entire value range.

TABLE I.

Demographic information of subjects with ASD and normal controls (NC) (age is in month)

Group \ Age	23	24	25	26	27
ASD (F/M)	6 (3/3)	37 (10/27)	9 (1/8)	5 (0/5)	3 (1/2)
NC (F/M)	23 (7/16)	128 (53/75)	44 (19/25)	13 (2/11)	3 (1/2)

TABLE II.

COMPARISON BETWEEN DIFFERENT METHODS IN TERMS OF DICE COEFFICIENTS (MEAN $\pm$ STD)

	Amygdala	CA1-3	CA4/DG	Subiculum
DRUNET	0.846 $\pm$ 0.008	0.827 $\pm$ 0.008	0.842 $\pm$ 0.012	0.801 $\pm$ 0.002
SegNet	0.834 $\pm$ 0.017	0.764 $\pm$ 0.021	0.705 $\pm$ 0.016	0.695 $\pm$ 0.005
U-Net	0.795 $\pm$ 0.006	0.720 $\pm$ 0.010	0.695 $\pm$ 0.018	0.674 $\pm$ 0.008
DDUNET	0.909$\pm$0.021	0.880$\pm$0.010	0.854$\pm$0.006	0.815$\pm$0.016