Imaging Analysis and Evaluation of Neural Stem Cell Intracerebral Migration and Functional Reconstruction Based on Deep Learning

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ABSTRACT Neural stem cell is a type of stem cell with self-renewal ability and multi-directional differentiation potential. Under certain conditions, neural stem cells can differentiate into neurons, oligodendrocytes, and astrocytes, thereby participating in the occurrence of the nervous system. Considering that deep convolutional neural networks have better feature learning capabilities for image data than feedforward neural networks, this paper studies how to apply deep convolutional neural networks to modeling based on imaging features, and constructs convolutional neural networks. In this paper, the distribution of CD133+ neural stem cells in different neuroanatomical regions of rat brain and possible migration flow were studied. The experimental results show that there are obvious differences in the distribution of neural stem cells in different neuroanatomical regions. With the growth and development of rats, a large number of CD133+ neural stem cells migrate from the subventricular zone to the surrounding ganglia, corpus callosum, and cerebral cortex. Seven days before the operation, the rats were trained in water maze, and the EL (Escape Latency) of the rats was recorded for 1 week, 2 weeks and 1 month. Compared with the control group of sham operation, EL was significantly increased in the cerebral ischemia-reperfusion group. Compared with cerebral ischemia-reperfusion + acupuncture group and cerebral ischemia-reperfusion group, EL was significantly smaller. The results show that electroacupuncture can induce the proliferation of newborn cells in the brain and promote the differentiation of newborn cells into glial cells and nerve cells. After electroacupuncture intervention, a small number of new nerve cells already have the activity and function of secreting Ach. Electroacupuncture intervention can promote the recovery of rat nerve function after cerebral ischemia and reperfusion.

INDEX TERMS Neural stem cells, migration flow, functional reconstruction, convolutional neural network.

I. INTRODUCTION NSC (neural stem cell) exists during the embryonic period and adulthood of mammals, and the regeneration, differentiation, and migration capabilities of neural stem cells are closely related to degenerative diseases of the central nervous system [1]. In recent years, researches on neural stem cell transplantation for the treatment of neurodegenerative diseases have been widely carried out and achieved good results [2]–[4]. With the in-depth exploration of science, through the application of histology, isotope tracing, retrovirus tracking, fluorescent staining labeling and modern imaging technology, it has been confirmed that under normal circumstances, the developing neurons must go through the migration process [5]. Only special parts can differentiate into neurons with special functions. The migration of neural stem cells is not only an indispensable stage of the normal development of the central nervous system, but also an essential foundation for the survival and neural function of neural stem cells [6], [7]. Therefore, it is of great significance to study the distribution and migration status of neural stem cells.

The development and regeneration of the central nervous system has always been a focus of research in the neuroscience community [8]–[10]. The traditional concept is
that adult mammalian brain tissue neurons are the ultimate cells [11], [12]. Once they mature, they will no longer proliferate and differentiate. After brain injury in adults, the phenomenon of losing nerve cells in the brain is also permanent [13]. The lost nerve cells can only be filled by glial cells. This traditional understanding has now been completely broken [14]–[16]. Relevant scholars have successfully isolated NSC from adult rat striatum using serum-free medium [17]. The cells of most cell types of the nervous system have the ability to respond to injuries and diseases, breaking the view that the adult central nervous system cannot be repaired after injury [18], [19]. Related research on the rhesus monkey cerebral ischemia model also showed that Brd U (5-bromo-2-deoxyuridine) -labeled positive cells increased in SVZ (Subventricular Zone) in the second week after transient cerebral ischemia [20]. Marker cells migrate along RMS and infarcts. Even with the cerebral ischemia model of old rats (24 months old), Brd U positive cell proliferation can occur in the SVZ zone, indicating that the old brain can also induce neurogenesis [21]–[23]. Relevant scholars injected the retrovirus with green fluorescent protein into gerbil DG (Deftlate Gyrus) [24], [25]. After 48 hours, the bilateral common carotid artery was occluded for 5 minutes to cause a transient cerebral ischemia model. It was found that almost all EGFP positive cells were present in SGL (Subgranular Layer), these cells proliferate and migrate to GCL (granule cell layer), expressing growing neuronal markers polysialic acid nerve cell adhesion molecules and microtubule-associated proteins of neuronal precursor cells, and differentiate into mature after 30 days Neurons suggest that cerebral ischemia promotes neurogenesis in the DG area [26]–[28]. In addition, it has been confirmed in the study of the gerbil transient cerebral ischemia model that after 4 weeks of ischemia, the immature neural precursor cells in the hippocampus SGZ (Subgranular Zone) area can be significantly transformed into mature neurons [29]. There are obvious new neurons in the cell layer. It is believed that neurogenesis in the pyramidal cell layer of the hippocampal CA1 area can promote the morphological and functional repair process after cerebral ischemia [30]. At present, it is generally believed that the enhancement of dentate gyrus neurogenesis may be a compensatory response to ischemic injury, which can promote the recovery of hippocampal function after ischemic injury [31], [32]. Studies have reported that inducing human-derived pluripotent stem cells into glial precursor cells and transplanting them into animal models of cerebral ischemia can improve the nervous system function of animals with cerebral ischemia injury [33]–[35]. Vascular endothelial growth factor secreted by glial cells can reduce ischemic damage to the nervous system [36]. Relevant scholars used morphological methods to detect MCAO (Middle Cerebral Artery Occlusion), rat ependymal, subependymal and choroid plexus cell proliferation, indicating that ependymal, subependymal and choroid plexus cell proliferation are also involved self-repair mechanism after cerebral ischemia [37]. At present, there are two ideas for repairing central nervous system damage using neural stem cells: the “replacement therapy” strategy of transplanting exogenous stem cells and the “supplemental therapy” strategy of activating endogenous stem cells [38]. Endogenous neural stem cells have the advantages of stable and reliable sources, no ethical and moral issues, no immune rejection, and no tumorigenicity. Therefore, how to fully mobilize endogenous neural stem cells to treat neurological diseases has become a new research hotspot. Related scholars used photochemical induction to make a cerebral ischemia model, and found that a large number of proliferating cells existed around the lesion 7d after cerebral ischemia, of which 3% −6% of proliferating cells differentiated into neurons and were randomly distributed in layer II–VI cerebral cortex [39]. And the density of new neurons around the ischemic focus is the highest.

At present, many studies have been made on the migration and distribution of neural stem cells, but there are few studies on the functional reconstruction of neural stem cells [40]–[42]. So far, there has been no comprehensive and in-depth detection of neural stem cells, migration flow, and neurogenesis signaling pathway regulation, and no one has conducted relevant experimental verification [43]–[45]. This paper explores the migration and functional reconstruction of CD133+ neural stem cells in the main distribution area of the brain and neural stem cells during neurogenesis. Specifically, the technical contributions of this paper can be summarized as follows:

First: In order to better analyze the images of neural stem cell migration and functional reconstruction, a convolutional neural network model was constructed. The model does not require pre-training on a large amount of auxiliary data offline in advance. It focuses on analysis of network parameter learning and convolutional neural network model pre-training.

Second: When a disease occurs in the central nervous system of the body, NSCs will specifically migrate to the lesion or injury site, and their new neurons replace the missing cells and establish pathways with other neurons, thereby causing damaged brain tissue. The research results in this paper allow us to consider that by promoting the regeneration and migration of neural stem cells, the diseased or injured nervous system can be treated. It is of great scientific significance to study the distribution and migration of neural stem cells. This topic can provide a new therapeutic idea for diseases related to the central nervous system.

Third: The effects of acupuncture intervention on neurogenesis and neural function recovery after cerebral ischemia-reperfusion in rats were analyzed. The role and possible mechanism of acupuncture intervention in neurogenesis and nerve function reconstruction after ischemic brain injury were explored to provide an experimental basis for acupuncture treatment of ischemic cerebrovascular disease. Studies have shown that intervention can promote the proliferation of neonatal cells after cerebral ischemia-reperfusion in rats, promote the differentiation of neonatal cells into nerve cells and glial cells, and promote the recovery of rat nerve function after cerebral ischemic injury.
The rest of this paper is organized as follows. Section 2 constructs a convolutional neural network model. Section 3 studies the migration of neural stem cells in the brain of rat brain. Section 4 investigates the neural function reconstruction of neural stem cell migration in the brain after cerebral ischemia and reperfusion in rats. Section 5 summarizes the full text and points out future research directions.

II. CONSTRUCTION OF A CONVOLUTIONAL NEURAL NETWORK MODEL

A. NETWORK STRUCTURE

For comparison, this section first briefly reviews the principles of a typical feedforward neural network model. The feedforward neural network is a series of hidden layers connected in series, and accepts a normalized vector data as the network input. The hidden layer of the feedforward neural network is composed of some neurons that perform nonlinear transformation processing, and each neuron is fully connected with the neurons of the front and back layers. The last layer of the feedforward neural network is usually also called the output layer, and its output type generally needs to be determined according to the specific task class (such as classification task or regression task).

In contrast, the convolutional neural network is another special neural network model. Different from the above feedforward neural network, convolutional neural network is usually composed of three types of hidden layers cross-connected with each other. The three types of hidden layers are convolutional layer, pooling layer and ordinary fully connected layer. Figure 1 shows the structure of the convolutional neural network.

Convolutional neural network is a multi-layer perceptron model specifically designed to simulate the human visual system. Its particularity is mainly reflected in the non-full connection (or sparse connection) between layers and the sharing of node weights in the layer. First, the sparse local connection between adjacent layers can better mine the spatial local characteristics in the image, that is, the cells in the mth hidden layer are only connected to the local area in the m-1 hidden layer, but not all connection. The lower layer simulates the function of visual nerve cells that only process smaller visual receptive fields, and as the number of layers continues to increase, the receptive fields of nodes in the higher layers gradually cover larger and even the entire image area. Second, each filter in the convolutional layer can cover the entire image field of view by sharing weights, and the unit composed of these shared weights is also called a feature map. The weight sharing mechanism greatly reduces the number of training parameters required in the model, and the visual features learned at the same time are not sensitive to the absolute position in the field of view, thereby extracting image features more efficiently. These characteristics make the convolutional neural network have better learning and generalization capabilities for visual tasks than ordinary feedforward neural networks.

B. CONVOLUTIONAL LAYER AND POOLING LAYER

The function of the convolutional layer is to use some learnable parameterized convolution kernels to perform a sliding convolution operation on the feature map output by the previous layer of the network to generate a new feature map. For the network input layer, the feature map can generally be a single-channel grayscale image or a 3-channel RGB image. Here, the convolution kernel is denoted as k, and its shape is usually a matrix with odd side lengths, such as 3 × 3 or 5 × 5. It should be noted that the odd side length is just for the convenience of the calculation process. In the convolutional neural network, the process of obtaining the feature map x_{ij} output by the convolution layer 1 through the convolution operation can be expressed as the following formula:

$$x_{ij} = f(b_{ij} + \sum_{i \in M_j} k_{ij} \cdot x_{i-2})$$

(1)

where M_j represents the set of input feature maps selected for convolution operations. In actual operation, M_j generally has multiple feature maps. For example, when three channels of an RGB image are simultaneously input to the convolutional layer as a feature map, M_j can be a set containing these three feature maps. k_{ij} is the convolution kernel used between the i-th input feature map and the j-th output feature map. b_{ij} is the offset coefficient corresponding to the j-th output feature map. f (·) is a nonlinear excitation function. In convolutional neural networks, the most commonly used is the ReLu (Rectified Linear units) function. The mathematical form of the ReLu function is very simple, as shown in the following formula:

$$ReLu(x) = \max(0, x)$$

(2)

Compared with the classical S-shaped growth function, the Re Lu excitation function can significantly increase the convergence rate of model parameters during training to greatly shorten the model learning cycle. In most existing deep convolutional network models, the Re Lu function basically replaces the traditional S-shaped excitation function.

The operation of the pooling layer will not change the number of input feature maps, but will reduce the size of the feature maps by a certain proportion. This scaling down pooling operation is similar to the image downsampling operation, so the pooling layer is also called a downsampling layer. At present, there are two main types of pooling layers: maximum pooling and average pooling. The main difference between the two pooling operations is whether the final pooled output value is the maximum or average value of each corresponding small area. The general form of pooling layer operations in convolutional neural networks can be expressed as:

$$x_{ij} = f[b_{ij} + \beta_j pooling(x_{i-1})]$$

(3)

Here, pooling (·) means a pooling operation similar to image downsampling. The usual pooling operation is to downsample the input feature map without overlapping, so the size of the output feature map is 1 / s of the size.
of the input feature map. The general form of the pooling layer is also equipped with an additive bias factor $b$, a multiplicative bias factor $\beta$, and a nonlinear processing function $f(\cdot)$, which are not necessary. Therefore, most of the current convolutional neural network pooling layer omits these parameters, that is, the pooling layer generally does not contain the parameters to be trained. Without affecting the understanding, the following discussion of the pooling layer uses simplified forms.

C. NETWORK PARAMETER LEARNING

A convolutional neural network usually also contains fully connected layers. This section will focus on how to use error back propagation to train the convolutional layer and pooling layer in the convolutional neural network. Similar to the feedforward neural network training method, the chain criterion based on the error sensitivity $\delta$ is still used here to derive the partial derivatives of each layer. However, the difference is that the calculation of the error sensitivity of the convolutional layer and the pooling layer in the convolutional neural network is slightly different and needs to be treated. The following takes the commonly used convolutional layer and pooling layer alternate connection form as an example to illustrate the network layer 1 training method.

When convolution layer 1 is followed by a pooling layer $l+1$, the error sensitivity of the jth output feature map of convolution layer 1 is calculated as follows:

$$\delta^l_j = f'(u^l_j) \cdot \beta^{l+1}_j \cdot \text{unpooling}(\delta^{l+1}_j)$$  \hspace{1cm} (4)$$

Among them, $\beta^{l+1}_j$ is a constant factor corresponding to the j-th feature map in the convolutional layer. The constant factor term can be omitted in the simplified model. Unpooling (·) is an anti-pooling operation or an upsampling function. Since layer 1 + 1 is a pooling layer, it is equivalent to downsampling the feature map output from layer 1 by a certain percentage. Therefore, during the back-propagation process, the error sensitivity of the layer $l + 1$ needs to be upsampled to make the size of the sensitivity map consistent with the size of the convolutional layer feature map. Taking the maximum pooling operation of size $s \times s$ as an example, the anti-pooling operation is to copy the value of $\delta^{l+1}_j$ directly back to each element in the $s \times s$ area. The value of $\delta^{l+1}_j$ is evenly distributed to each element in the $s \times s$ area. In addition, it is worth noting that the de-pooling operation can also be achieved more efficiently by the direct product of the matrix unpooling $(x) = x@s \times s$. Regarding the nonlinear excitation function $f(\cdot)$, when the ReLu function is used, its partial derivative form is shown in the following formula, where $l(\cdot)$ is an illustrative function.

$$f'(x) = l, \quad \text{ReLu}(x) \geq 0 \quad (5)$$

For the error sensitivity calculation of a pooling layer 1 followed by a convolutional layer $l+1$, here we use a simplified pooling layer with no parameters to be trained. Each feature map i of the pooling layer corresponds to its own error sensitivity, and its value is the sum of the contributions of all convolution kernels in the convolutional layer, as shown in the following formula:

$$\delta^l_i = \sum_{j=1}^{k^{l+1}} k^{l+1}_j \cdot \delta^{l+1}_i \hspace{1cm} (6)$$

After the error sensitivities of the convolutional layer and pooling layer feature maps are obtained separately, the partial loss derivative of the target loss function for the convolutional layer 1’s bias $b^l_j$ can be calculated (here the pooling layer has no parameters to be trained), as shown in the following formula:

$$\frac{\partial E}{\partial b^l_j} = \sum_{u,v} (\delta^l_j)_{uv} \hspace{1cm} (7)$$

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Similarly, the partial derivative of the convolution kernel parameter with respect to the target loss function can be expressed as:

$$\frac{\partial E}{\partial k_{ij}} = \sum_{u,v} (p_{ij}^{l-1})_{uv} (\delta_i^l)_{uv}$$  \hspace{1cm} (8)$$

Here, the parameter to be trained in the convolutional layer is the convolution kernel $k_{ij}$. Among them, $(p_{ij}^{l-1})_{uv}$ is a sub-region in the $x_i^{l-1}$ of the l-1 layer feature map, which is multiplied point by point with the convolution kernel $k_{ij}$ and generates the feature value at the position $(uv)$ of the $x_i^l$ of the l layer feature map.

### D. PRE-TRAINING OF CONVOLUTIONAL NEURAL NETWORK MODELS

The pre-training of CNN is divided into two steps, forward propagation and error back propagation. Suppose that the CNN network is used to process n training samples of m types, and the single input sample is $(x(i), y(i))$, where $x$ (i) is the input vector of R dimension, and the form of the input vector is converted into the length and width of the image, and $y$ (i) is the true category label corresponding to this sample. Let l denote the current layer, the input feature map of layer 1 is $x^{(l-1)}$, and the output feature vector is $x_1$, and a sliding convolution filter of this layer takes the value $w_1$ and the offset $b_1$.

In the forward propagation process, the output of each layer is calculated by the input, and the calculation method is:

$$x^l = f(z^l), \quad z^l = b^l + w^l x^{l-1}$$  \hspace{1cm} (9)$$

For a data set containing n samples, the CNN model cost function is:

$$J(w, b) = \frac{\lambda}{2} \sum_{l=1}^{n_l-1} \sum_{i=1}^{s_l} (w_{ij}^l)^2 + \frac{1}{n} \sum_{i=1}^{n} f_w,b(x^{(i)} - y^{(i)})$$  \hspace{1cm} (10)$$

Among them, $\lambda$ is the weight penalty term, $n_l$ is the number of layers of the network, $s_l$ is the number of neurons in the lth layer of the network, and the random batch gradient descent method is used to optimize the network parameters to minimize the overall cost function of the network. The chain derivation rule is used to update the weight and offset of each layer.

When the error loss of the entire network is minimum, a pre-trained optimization model is obtained.

The error back propagation process uses BP algorithm to adjust the parameters. For the high-level statistical features output by the fully connected layer, a Softmax classifier is supervisedly trained to classify breast cancer benign and malignant dataset images. In order to improve the accuracy of the classifier, and at the same time continue to propagate the training residuals, the BP algorithm is used to supervise the fine-tuning of the adjacent base volume layer before the fully connected layer.

The parameter adjustment of the BP algorithm is mainly to calculate the residual $\delta_i^l$ of each output node of each layer, which indicates how much influence the node has on the error of the final output value. The Softmax classification used in the final classifier layer in this paper, the deviation of each output neuron of the Softmax classifier is expressed as:

$$\delta_i^l = f'(\eta_i^l) \cdot (y_i - x_i^l)$$  \hspace{1cm} (11)$$

The residual of the back layer propagates back to the front layer, then the residual of the front layer is defined as:

$$\delta_i^l = p' (h_i^l = 1 | v, \theta) \cdot \delta_{i+1}^l$$  \hspace{1cm} (12)$$

The traditional Sigmoid function is used as the activation function of the neural network. When the gradient is propagated back from the output layer, the error in each layer must be multiplied by the input neuron value x of the layer and the first derivative of the activation function. The function will have derivative scaling and saturation scaling issues. In this case, the error back-propagation will be doubled when passing through each layer of neural network. When deep back-propagation occurs, the gradient will disappear, making the network learning slower. The correct activation function (Relu) has only one end saturated and the gradient is 1. The gradient can be quickly transferred in the back propagation and accelerate the speed of model convergence. Therefore, ReLu is used as the activation function in the convolutional layer.

### III. MIGRATION OF NEURAL STEM CELLS IN THE BRAIN OF RAT BRAIN

#### A. CUBIC METHOD

CUBIC (Clear Unobstructed Brain / Body Imaging Cocktails) is an efficient method to make organ tissue transparent. The transparentized samples were analyzed by Lightsheet Z.1 laser slice scanning microscopy system and quantitative imaging. The general organ or tissue transparency process takes about 15 days. The transparency time is determined by the experimental sample itself and the purpose of the experiment. This method can easily and quickly acquire imaging information for the entire organ or tissue. The subject of this experiment is to take rats of different periods as the experimental object, and then conduct 3D imaging after transparency treatment.

After the tissue or organ is transparent, 3D imaging is a method that can analyze multiple cells in the same organ or tissue. The development of this technology has become the future trend. In the early stage of research and development, tissue transparency 3D imaging mainly used some organic reagents for liquidation and transparency. Some of the technical methods are to remove the lipid of the organ to make the organ transparent within a few days, and then balance its refractive index. During the balance of the refractive index, immunofluorescence staining can be performed. However, the quenching and safety of fluorescent proteins require further exploration of this technology. At present, there are some technical methods to reduce the quenching...
degree of fluorescent protein by controlling the pH and temperature during the experiment during the transparency process, and the protection of fluorescent protein is also very effective. The scale method uses the hydrophilic reagent urea, the See DB method, the Clear method and other organic reagents. The disadvantage is that the transparency effect is relatively low. The transparent method used in this subject is the CUBIC method. This method can efficiently remove lipids in organs or tissues to make it transparent to the greatest extent. During the experiment, the accuracy of the instrument type is relatively low, and it can also protect the target fluorescent protein. CUBIC can make the whole organ or the whole experimental animal transparent. In this project, the rat brain is used for 3D rapid imaging using CUBIC transparency, and the 3D imaging information of the entire brain is extracted for data analysis.

When developing and applying the CUBIC method to transparentize the whole brain, two main criteria are considered. First, it is the efficiency and transparency of the processing. When using the Zeiss Lightsheet Z.1 laser slice scanning microscopy system for 3D imaging, it can quickly Imaging; the second is good fluorescence reproducibility, which can be used for comparative analysis of multiple samples. Based on these requirements, the CUBIC method has been continuously improved and optimized. The CUBIC method selected for this project mainly requires the preparation of two reagents, Scale CUBIC-1 (Reagent 1) and Scale CUBIC-2 (Reagent 2). This method can effectively reduce the scattering of light in the tissue and avoid reducing the fluorescence intensity. The main role of the first reagent is to remove lipids. Lipids are the main light scattering material inside tissues, and the degree of removal is closely related to transparency. After the fixed rat brain reagent was processed for about a week, the buffer solution washed the reagent one and then immersed in the reagent two. The entire rat brain processing cycle is about 15 days. Due to its efficiency and reproducibility, the CUBIC method is suitable for processing multiple samples simultaneously. In addition, this method can stain the entire organ with nuclear dye to track the target cells, and can also compare the signal strength between different samples. In short, the CUBIC method provides a platform for comprehensive analysis of target cells in the entire organ.

B. EXPERIMENTAL SAMPLE PREPARATION

Before the experiment begins, we need to identify the genotype of the rat to ensure the accuracy of the experiment. This subject mainly detects whether Zs Green rats and CD133 rats are homozygous genotypes to ensure that the genotypes of rats are CD133 × Zs Green.

The amount of Tm (Tamoxifen) has a great influence on the individual health of rats. Too much injection will cause death or miscarriage in rats. Too little injection will not play a good role. In addition, after Tm injection, the sampling time of the rat brain has a great influence on the experimental results. If the expression of the green fluorescent egg is too small, the expected result cannot be observed, and if the sampling is too late, the excessive green fluorescent protein affects the experimental result.

The amount of Tm injected into rats must be determined through experiments to ensure the best amount and time of experiment.

1) We select CD133 or Zs Green rats with a gestation period of 15.5 days to inject Tm, the injection volume is 10ul / g. Three days later, the rats were injected for the second time with an injection volume of 10ul / g. As a result, it was found that some rats had miscarriages that could not be delivered normally; some rats found more oil in the body when taking rats.

2) We select CD133 or Zs Green rats with a gestation period of 15.5 days to inject Tm, and only inject Tm once, with an injection volume of 10ul / g, and select rats for 5 days for comparison. There were fewer accidents of stillbirth in rats under this injection volume.

C. ZEISS LIGHTSHEET Z.1 IMAGING

After the rat brain was transparentized by CUBIC method, 3D imaging was carried out with ZEISS Lightsheet Z.1 laser slice scanning microscopy system. The imaging system uses an optimized ultra-microscope head. Each rapid imaging can only process a single transparent brain. It takes about 30 to 60 minutes for each dye to complete imaging. The fluorescence wavelength of the sample will affect the imaging effect. Compared with green fluorescence, red light can penetrate a thicker tissue layer to obtain a better imaging effect. This difference will be more pronounced for thicker tissue layers. The imaging system is that both sides of the laser can image the sample at the same time, which ensures that the weak fluorescence signal in the deep layer can be detected.

Image visualization software Imaris can be used for 3D imaging reconstruction. Imaris implements many image analysis functions, including point counting and surface feature extraction.

After the transparent treatment, the rat brain was immersed in the reagent two, and before the imaging, the air bubbles in the reagent two and brain tissue were removed with an ultrasonic breaker to avoid affecting the experimental results. After removing air bubbles, the sample is placed on the sample holder for imaging. During the imaging process, you pay attention to the absence of bubbles in reagent 2. The imaging thickness and width of the sample should be set appropriately. You move the sample slowly to prevent the sample from falling off from the sample holder or generating bubbles, set the relevant parameters of the Lightsheet.1 imaging system for imaging processing.

D. ANALYSIS OF EXPERIMENTAL RESULTS OF NEURAL STEM CELL MIGRATION IN THE BRAIN

1) DOSAGE AND TIME OF TAMOXIFEN INJECTION IN EXPERIMENTAL ANIMALS

Rats were used as experimental subjects to inject Tm, and the injection volume of Tm was 10ul / g. The Tm concentration...
FIGURE 2. Activity curves of neural stem cells with different concentrations of Tm.

FIGURE 3. 15.5 + 5 Horizontal section Zs Green cell distribution results.

Comparing the distribution of Zs Green cells in the brains of rats with different Tm injection lengths, it was found that the Tm injection duration was 3 days, and the best results were obtained. Therefore, the subsequent CUBIC method selected the rats with Tm injections for 3 days for Lightsheet z.1 three-dimensional imaging. In order to further ensure the correctness of the selected period, the subject also performed three-dimensional Lightsheet z.1 imaging of rats injected with Tm for 5 days.

During the test, it was found that the Zs Green cells migrated between the third ventricle and the hypothalamus, but the migration direction was not clear; the Zs Green cells around the lateral ventricles also migrated to the surrounding ganglia, corpus callosum, and cortex migrate.

2) LIGHTSHEET Z.1 LASER SLICE SCANNING MICROSCOPY 3D IMAGING RESULTS

The rats were selected in the 15.5 + 5 period, and the Lightsheet Z.1 laser slice scanning microscopy system was imaged. It was found that Zs Green cells still migrate quickly, and there is no visible RMS migration flow. However, a large number of Zs Green cells are distributed in the fourth ventricle and the upper cerebral duct. It is not clear why these cells gathered in the rat period. This area may be related to the development of the cerebellum and prepare for postnatal movement. In addition, other staff of this subject performed 3D imaging on the rats 7 days after birth, and they can see the obvious RMS migration flow, which indicates that the RMS migration flow has not occurred or migrated less during the rat period.

Subsequently, the duration of Tm injection was shortened, and 15.5 + 2 rats were subjected to 3D imaging with Lightsheet Z.1 laser slice scanning microscopy system, and no obvious RMS was also found. The cells still migrated quickly. The changes in the degree of migration of neural stem cells in the brain over time are shown in Figure 4.

In the research of this subject, the main distribution area of CD133 + neural stem cells was explored by immunofluorescence staining and three-dimensional imaging, and the
migration direction of some neural stem cells was identified. The relevant experimental results can provide some reference for the treatment of neurodegenerative diseases.

IV. RECONSTRUCTION OF NEURAL FUNCTION OF NEURAL STEM CELLS IN BRAIN MIGRATION AFTER CEREBRAL ISCHEMIA-REPERFUSION IN RATS

A. PREPARATION OF MCAO MODEL IN ADULT RATS

Zea-longa thread embolism method was used to improve the model of focal cerebral ischemia-reperfusion. Rats were given intraperitoneal administration of 10% chloral hydrate at a dose of 0.3ml/100g. After anesthesia, they were fixed on the operating table in a supine position. They were sterilized by conventional methods, and the right side of the neck was incised to expose and separate the right CCA (Common Carotid Artery), ICA (Internal Carotid Artery) and ECA (External Carotid Artery). Non-invasive microvascular clips clamp the CCA, ICA and branched pterygopalatine arteries, ligate the ECA, cut a small slit at the proximal end of the ligation, insert the nylon plug pre-soaked in heparin saline from the opening, loosen the microvascular clip. The nylon embolus enters the ICA through the CCA. When there is a slight sense of resistance, it indicates that the embolus reaches the front end of the ACA (Anterior Cerebral Artery) and crosses the beginning of the MCA (Middle Cerebral Artery), which completely blocks the MCA blood flow, causing focal cerebral deficiency. The blood state is marked by the bifurcation of ECA and ICA, and the general insertion depth is \((18 \pm 0.5) \text{ mm}\). In the sham-operated experimental control group, the thread was inserted only about 15 mm after the blood vessel was separated.

In the process of making animal models, the animal is always kept under light anesthesia with 10% chloral hydrate, that is, there is a sensitive corneal reflex, and the tail clip can cause leg flexion, but the animal does not have any spontaneous activity. During the operation, incandescent lamps were used to maintain the animal’s anal temperature at \((37 \pm 0.5) ^\circ \text{C}\). Chloraldehyde hydrate should be used and prepared immediately, and discarded after 6 hours. During anesthetizing animals with chloral hydrate, rats are prone to increased respiratory secretions. Care should be taken to clean the respiratory tract in time. Once sputum asphyxiation symptoms occur, they are eliminated. The wire should be properly handled, or slightly soaked with nitric acid to soften, or the front end of the wire should be lightly burned, or the front end of the wire should be lightly dipped...
with a small amount of rat glue or thin nail polish to reduce the puncture of cerebral blood vessels to cause subarachnoid hemorrhage complications. When the thread insertion reaches about 18mm, close attention should be paid to the change of the thread insertion resistance to reduce complications such as subarachnoid hemorrhage and improve the success rate of the model. Timely you make up the number of animals that died during the animal experiment was rejected by the inspection. The animal mortality rate and model failure rate during the preparation of the animal model in this study are relatively high. Therefore, the number of animals listed in each test item described in this paper is the number of animals that have succeeded in the final model.

After the animal was naturally awake for 1h (approximately 3h after surgery), the rats were scored for neurological function according to Zea-longa’s grade V scoring standard. Rats scoring grades II to IV were model qualified animals, rats of grade III do not meet the requirements and are not used for elimination (rats with neurological dysfunction score of V grade are often accompanied by complications such as subarachnoid hemorrhage). Each experimental group was sacrificed according to the experimental design at the corresponding time point after cerebral ischemia and reperfusion. The samples were carefully checked for subarachnoid hemorrhage. Those with subarachnoid hemorrhage were excluded and no subarachnoid space was used. Hemorrhage continues to the next experiment.

Rats were intraperitoneally anesthetized with 10% chloral hydrate, thoracotomy, left ventricle intubation, and full saline perfusion. 2% TTC was first infused with 100ml quickly (20 drops / min) and then slowly (5 drops / min). They were incubated for 1 hour in a 38 °C humid and hot incubator, and then fixed with 4% paraformaldehyde left quickly (20 drops / min) and then slowly (5 drops / min). They were incubated for 1 hour in a 38 °C humid and hot incubator, and then fixed with 4% paraformaldehyde left ventricle perfusion and fixation. After taking the material, we fix with 4% paraformaldehyde for 6 hours, slice the frontal plane of the brain, slice thickness 2mm. Cerebral ischemic infarcts are pale and non-infarcts are red.

B. DETECTION OF NEWBORN CELLS AND NESTIN PROTEIN ON THE SIDE OF CEREBRAL ISCHEMIC INJURY

1) DETECTION OF NEONATAL CELLS ON THE SIDE OF CEREBRAL ISCHEMIC INJURY

Flow cytometry detection technology is a high-tech technique for quickly measuring the physical and chemical properties of cells. It has the advantages of fast speed, high precision, and good accuracy. It is one of the modern advanced cell quantitative analysis technologies. Brd U is an analog of DNA precursor thymidine and can compete with it in cellular DNA synthesis and be incorporated into newly synthesized DNA molecules in cells. In this experiment, flow cytometry was used to detect Brd U that can specifically bind to anti-Brd U antibody on the single-stranded DNA molecule of the cell to conduct cell proliferation research, and MFI (Mean Fluorescence Intensity) was used to indicate cell proliferation. The specific operation flow is shown in Figure 5.

2) DETECTION OF NESTIN PROTEIN ON THE SIDE OF CEREBRAL ISCHEMIC INJURY

We place the specimen in a mortar filled with liquid nitrogen and quickly grind it, add 2ml of cell lysate and stir, then transfer to a centrifuge tube, add 2 × loading buffer, cook in a 95 °C water bath for 10 minutes, then take out the centrifuge tube and immediately put it into crushed ice for partial filling, and store at 80 °C until use.

After electrophoresis, the corresponding separation gel was cut and stained with R250, and the target protein was determined according to the marker of the protein marker. We remove the gel from the electrophoresis tank, carefully peel off the separation gel, lay it flat on the filter paper, cover the PVDF membrane, transfer the membrane in a chromatography cabinet at 4 °C, 50mA, overnight.

We mix the two reagents A and B in the ECL (Electro-Chemical Luminescence) kit on the cling film, and make the PVDF membrane protein face down to fully contact this mixture; after 1 min, we remove the residual liquid and move the PVDF membrane to put it on the other plastic wrap, put it in the X-ray film holder, the exposure time is generally 1 ~ 5min.

Laser confocal scanning microscopy was used to observe dual-channel tomography of immunohistochemical fluorescent double-labeled brain slices, while scanning Cy3-labeled positive reactants and FITC (Fluorescein Isothiocyanate) -labeled positive reactants. Scanning parameters:

Cy3 excitation light is green with a wavelength of 543nm; observation light is red with a wavelength of 590 / 70nm. The FITC excitation light is blue with a wavelength of 488nm. The observation light is green with a wavelength of 515 / 30nm. The scanning method is point scanning. Zoom is 1.0, and the image is saved as 512 × 512 pixel type. The microscope objective used for image acquisition is a 60x oil lens, which detects and acquires images.

C. ANALYSIS OF EXPERIMENTAL RESULTS OF NEURAL STEM CELL FUNCTIONAL RECONSTRUCTION

1) CHANGES IN PROLIFERATION OF NEONATAL CELLS ON THE SIDE OF CEREBRAL ISCHEMIC INJURY IN RATS

The results of flow cytometry showed that the number of new cells increased significantly 10-15 days after cerebral ischemia-reperfusion injury. Compared with the sham operation control group, the difference was statistically significant (P < 0.01). Among them, 15 days after cerebral ischemia-reperfusion, MFI reached a peak, compared with 1 day-14 day after cerebral ischemia-reperfusion, the difference was statistically significant (P < 0.01). Figure 6 shows the changes in the proliferation of newborn cells in the brain at different times after cerebral ischemia and reperfusion.

After 15 days of cerebral ischemia and reperfusion, MFI was also enhanced after acupuncture intervention compared with 15 days of cerebral ischemia and reperfusion alone. It shows that acupuncture intervention can promote cell proliferation after brain injury and increase the
number of new cells. Figure 7 shows the changes in the proliferation of new cells in the brain after acupuncture intervention.

2) EFFECT OF ACUPUNCTURE INTERVENTION ON THE EXPRESSION OF NESTIN PROTEIN ON THE SIDE OF CEREBRAL ISCHEMIC INJURY

Nestin protein is an intermediate silk protein. The expression in nerve cells starts in the embryonic stage. When the migration of nerve cells is basically completed, its expression begins to decline, and stops expression as the nerve cells mature. Nestin protein is mostly found in early primitive differentiated cells. Western blot detection in this experiment showed that the expression of Nestin protein in the cerebral ischemia-reperfusion group was stronger than that in the sham operation control group, indicating that cerebral ischemia can induce the proliferation of NSC on the side of cerebral ischemia injury. Nestin protein expression is enhanced, as shown in Figure 8. This shows that acupuncture intervention can enhance the proliferation of NSC on the ischemic injury side after cerebral ischemic injury. The prediction curve of the convolutional neural network of the relationship between Nestin protein expression and healthy survival of rats is shown in Figure 9.

3) EFFECT OF ACUPUNCTURE INTERVENTION ON INTRACEREBRAL NEUROGENESIS AFTER ACUTE CEREBRAL ISCHEMIA AND REPERFUSION IN RATS

Immunohistochemical staining showed that the nucleus of neonatal cells was blue and black, and immunofluorescence Cy3 staining showed that the nucleus of neonatal cells was red fluorescence. In the sham operation control group, there
were occasional neonatal cells in the cerebral cortex and striatum, and a small amount of neonatal cells in the choroid plexus. Compared with the sham-operated experimental control group, the neonatal cells in the ischemia-reperfusion group increased significantly, and showed a significant "clustering" phenomenon. In addition, there are more expressions in the cerebral cortex and meninges, and the expression of new cells increased significantly after electroacupuncture intervention. The number of Brd U positive cells in the lateral ventricle area is shown in Figure 10.

The number of Brd U positive cells in the cerebral ischemia-reperfusion group increased significantly compared with the sham operation control group (P <0.01), suggesting that cerebral ischemia can induce the proliferation of new cells on the ischemic injury side. The number of Brd U positive cells in the electroacupuncture treatment group was significantly higher than that in the cerebral ischemia-reperfusion group (P <0.01), showing that electroacupuncture treatment can promote the proliferation of new cells on the ischemic injury side, as shown in Figure 11.

FIGURE 11. New cells in the lateral ventricle area after cerebral ischemia 2h and reperfusion 7d.

FIGURE 12. Swimming trajectory of rat neural stem cells after functional reconstruction.
In this study, the experimental method of positioning swimming in the water maze of rats was used to place the rats from quadrant III into the water to reach a safe platform for EL. As shown in Figure 12, quadrant I is set as the target quadrant, and a safety platform is placed in quadrant I. Among them, A, B, C, D are sham operation control group, E, F, G, H are cerebral ischemia reperfusion group, I, J, K, L are cerebral ischemia reperfusion + acupuncture group; A, E, I is preoperative, B, F, J is 1 week after surgery, C, G, K is 2 weeks after surgery, D, H, L is 1 month after surgery.

The results of the water navigation labyrinth navigation experiment showed that the cerebral navigation ischemia-reperfusion group had a positioning navigation latency of 31.92 ± 4.05 s, 25.40 ± 4.26 s, and 22.16 ± 4.03 s respectively after 7 days, 14 days, and 1 month. The control group was significantly prolonged (P < 0.01). This suggests that cerebral ischemia and reperfusion can significantly inhibit the nerve function of rats, and the nerve function of rats can be improved to a certain extent as the reperfusion time is prolonged. In the electroacupuncture treatment group, the positioning voyage latent periods were 23.79 ± 4.40 s, 18.58 ± 2.51 s, 16.13 ± 1.72 s respectively after 7 days, 14 days, and 1 month, which was significantly shorter than the cerebral ischemia-reperfusion group (P < 0.01). This suggests that electroacupuncture intervention can restore the neurological damage in rats after cerebral ischemia-reperfusion injury. The changes of the escape latency of water maze in rats after electroacupuncture treatment are shown in Figure 13.

In this experiment, through immunohistochemical double-labeling, it was found that Brd U positive cells in the striatum of the injured side express a small amount of Ach after cerebral ischemia, indicating that these new brain cells already have the activity and function of secreting Ach. However, whether these new cells really have the function of nerve cells to promote the recovery of brain function still needs further study. Therefore, it is necessary to further clarify the neurogenesis mechanism and regulatory factors after cerebral ischemic injury, use acupuncture and other means to expand the role of endogenous NSC, promote its proliferation and migration, and induce its differentiation into functional neurons.

V. CONCLUSION

In view of the huge advantages of convolutional neural networks in image processing, this paper proposes a modeling method based on multi-layer convolutional neural networks. A processing method for extracting training samples with high reliability and pertinence is designed. Three-dimensional imaging and immunofluorescence staining results showed that rat CD133+ neural stem cells migrated from the SVZ area to the surrounding ganglia and cerebral cortex. During the migration process, CD133+ neural stem cells differentiated into other types of cells. CD133+ neural stem cells in the ependymal region of the third ventricle migrated to the surrounding hypothalamus and differentiated. The rat model of focal cerebral ischemia-reperfusion was prepared by thread embolism method to observe the proliferation of neonatal cells, the location of neonatal cells and the nature of neonatal cells in the rat brain. The function of the new cells, the changes of nerve function after cerebral ischemia and the intervention effect of electroacupuncture were observed. We study the pathophysiological mechanism of acute focal cerebral ischemia-reperfusion and observe the changes of nerve function in rats, and provide further experimental basis for acupuncture treatment of ischemic cerebrovascular diseases. The results show that electroacupuncture can induce the proliferation of newborn cells in the brain, and the newborn cells can differentiate into glial cells and nerve cells. After electroacupuncture intervention, a small number of new nerve cells already have the activity and function of secreting Ach. Electroacupuncture intervention can promote the recovery of rat nerve function after cerebral ischemia and reperfusion. Due to the long processing time of the experimental sample, the next step is to make the sample transparent by the CUBIC method, and use the CD133+ protein antibody to perform fluorescent staining of the entire brain, so as to lock all areas and migration directions of CD133+ cells. In addition, whether new cells really have the function of nerve cells, and how to establish a functional relationship with the original nerve cells to promote the recovery of brain function still need further study.

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