XVI IMEKO World Congress
Measurement - Supports Science - Improves Technology - Protects Environment ... and Provides Employment - Now and in the Future
Vienna, AUSTRIA, 2000, September 25-28

VISUAL FEEDBACK SYSTEM FOR NEUROANATOMY

J. Sakiyama[1)], M. Okamoto[1)], H. Yamamoto[2)]
1) Faculty of Health Sciences, Okayama University Medical School
2-5-1 Shikatacho, Okayama 700-8558, Japan
2) Faculty of Education, Okayama University, Tsushima, Okayama 700-8530, Japan

Abstract: In this paper, we propose a visual feedback system for a neuroanatomy. The stereoscopic microscope image of the dentate gyrus of adult rat brain in the neuroanatomy is converted into a black and white image. By the region segmentation, the region of the dentate gyrus is extracted. A binary image is obtained from a microscopic photo of cresyl violet-stained coronal section of the rat brain as a template. By the template matching, the apex of the dentate gyrus is automatically detected. The visual feedback system consists of a stereoscopic microscope, a micromanipulator, two CCD cameras and a personal computer. According to the stereo vision method, the distance between the dentate gyrus and the tip of microelectrode is three dimensionally measured based on the position difference between the left and the right image. Following an insertion of the tip of microelectrode to the dentate gyrus, the lypophilic fluorescent tracer is slowly injected by a nanoinjector. As a result, the glass microelectrode can be guided to pierces the apex of the dentate gyrus in desired depth.

Keywords: Image Processing, Micromanipulation, Neuroanatomy.

1 INTRODUCTION

Visual image processing is widely performed in clinical and experimental medicine. Computer-based morphological analysis of neurons is an indispensable technique in neuroscience [2) - 7)]. Advances of gene therapy and neuronal transplantation in the treatment of neuronal diseases prompt us to develope a technique to identify the target brain lesions or neurons and inject the gene carriers or implant into them. In neuronal implantation, for instance, it is necessary to precisely inject neuronal cells into the target brain regions and analyze neurite extension or electrophysiological properties of implanted neurons. In some cases, it may be necessary to inject chemoatractants or neurotrophic factors into locations with exact distance from the implanted neurons or regions to where growth cones of axons or dendrites are introduced. In injection of genes and neuronal implants or in electrophysiological studies, the target brain regions or cells must be identified of an unfixed, unstained brain slice based on differences in reflection or absorbance of light of various brain structures. Although these experimental manipulations are usually done by manual under microscopic visual identification of the target regions or cells, computer-based visual image processing may help them especially when chronological changes of neuronal morphology are analyzed [3)].

Micromanipulation is widely used for electrophysiological and morphological analysis of cultured neurons and neurons of brain slices. However its general use is limited because sophisticated techniques and much burdens are required to operate micromanipulator. Therefore, an automated, easy-to-deal device is desired especially in the cases when electrical activities are recorded from the two or more neurons or neuronal populations separated at exact distance, or when drugs and tracers are injected into them [2)].

The hippocampus is a prominent brain structure which plays an important role in memory and learning. It's characteristic pathology in Alzheimer type dementia and temporal lobe epilepsy is well known. The hippocampus is an adequate brain structure for visual image processing and micromanipulation because it's cytological structure is simple and it's intrinsic and extrinsic neuronal connections are extensively studied [1)]. Here, we propose a visual feedback system for automated micromanipulation to inject fluorescent dyes, DiI and DiD in the rat brain slice by identifying the target hippocampal region (apex of the dentate gyrus) using visual image processing. Lypophic fluorescent dyes have advantages for neuronal tracing because they can trace neuronal processes of fromalin-fixed tissue and maintain their fluorescence after paraffin embedding procedure [5) - 7)].

2 IMAGE PROCESSING FOR TEMPLATE MATCHING

A coronal slice of adult Sprague-Dawley rat brain of (500 μm in thickness) is placed in a recording chamber filled with phosphate-buffered saline. The stereoscopic microscope color image of the hippocampus is converted into a black and white image and the noises are eliminated by a median filter. Fig. 1 shows a median-filtered image of the dentate gyrus of the coronally-sectioned rat brain.

The brain is sliced coronal 500 μm in thickness. The threshold for binary image is determined by the mean concentration and the standard deviation calculated from the region of the dentate gyrus. By the region segmentation, the area of the dentate gyrus is extracted. In the brain slice shown in Fig. 1, the mean concentration is 191.75 and the standard deviation is 4.40, respectively. Fig. 2 shows the dentate gyrus after region segmentation. However, many noises are emerged in the binary image. The

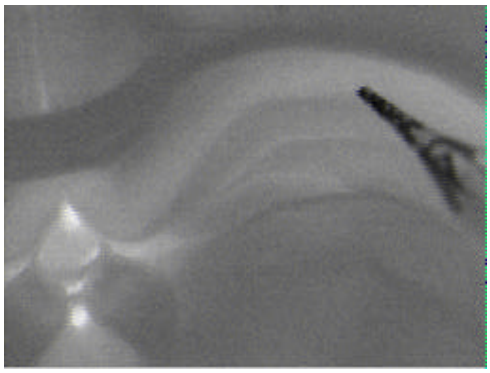

Figure 1. Dentate gyrus of rat brain.

Figure 2. Dentate gyrus after region segmentation

noises in the binary image are reduced after the dilation-erosion processing as shown in Fig. 3. Fig. 4 shows the extracted area of the dentate gyrus. In this image, the small regions, which are concerned as noises, are eliminated. The angular shaped granular cell layer of the dentate gyrus is clearly visualized because the dentate granular cell layer is large enough compared to small noises dispersed in hilus and molecular layer of the dentate gyrus, and CA3.

3 DETECTION OF THE APEX OF THE DENTATE GRANULAR CELL LAYER BY TEMPLATE MATCHING

For successful automated micromanipulation, an apex region of the dentate gyrus that is shown in Fig. 4 should be labeled with the template image. Fig. 5 shows a binary image of the template. At first, the template is made from microscopic photos of cresyl violet-stained coronal section of the rat brain. The area of the dentate gyrus is selected from microscopic photo image. A clearly defined image of the dentate gyrus is obtained by this processing because the granular cells are strongly stained with cresyl violet compared to adjacent molecular layer and hilar region. It is suitable for the template because the images which reflected morphological characteristics of the dentate gyrus. Secondarily, the template is sequentially scanned over the image in parallel translation and the common region between the template and the image is compared for similarity.

We estimate the correlations between the template and the image for the comparison of the similarity. The location of the template where the correlation became maximum value is detected. The apex is detected as shown in Fig. 6. Finally, the template is scanned over the image in rotational translation. As the same method, the angle of the apex is detected as shown in Fig. 7. In this case, the maximum correlation coefficient is 0.405873 when the center of the template image is located at i=249 and j=290 in the image as shown in Fig. 7.

Figure 3. Dentate gyrus after dilation-erosion processing

Figure 4. Extracted area of the dentate gyrus

Figure 5. The template of the apex of the dentate gyrus.

4 INJECTION OF LYPOPHILIC TRACERS INTO THE DENTATE GRANULAR CELL LAYER BY AUTOMATED MICROMANIPULATION

For automated micromanipulation, visual feedback system developed by Yamamoto and Sano[8)] is used. The system consists of a stereoscopic microscope, a micromanipulator, two CCD cameras and a personal computer. A glass microelectrode attached to the micromanipulator is filled with lypophilic fluorescent tracer and connected to a microinjector with a teflon tube. A paraformaldehyde-fixed coronal rat brain slice is placed in a recording chamber to the section of dorsal side of the brain upside, and the slice is fixed on the floor of chamber by a L-shaped glass rod. Lypophilic fluorescent tracer is injected into the apex of the dentate gyrus determined by image processing and template matching as described above. The position of the target and the tip of glass microelectrode are measured three-dimensionally by using two CCD cameras which are set on the eyepieces of microscope. The distance between the target and the tip of microelectrode is measured based on the position difference between the left and the right image, focal distance of the cameras, and the distance between the cameras. When the tip of microelectrode is closest to the target, the distance is approximated by the position difference and the constants determined from the position difference and the moving length of the tip. In order to carry out micromanipulation at a realistic rate, image data compression is used to reduce the image processing time for detecting the target and the tip of microelectrode. Following a successful insertion of the tip of microelectrode, the

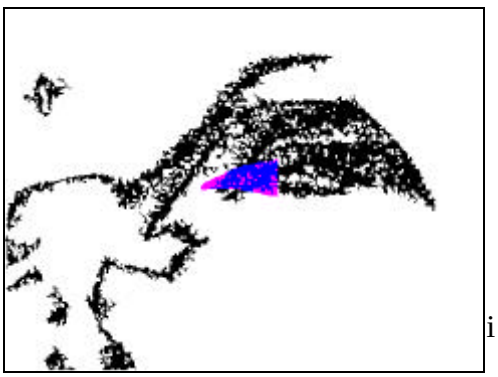

Figure 6. Parallel template matching.

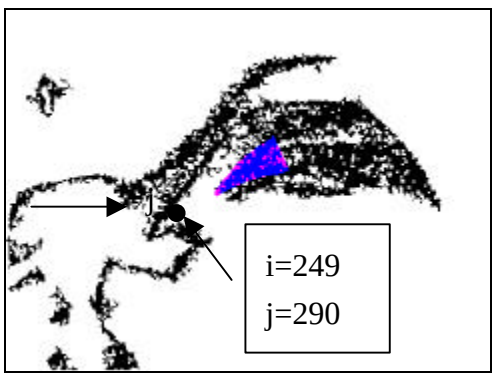

Figure 7. Rotational template matching.

Figure 8. The detected triangular-shaped apex of the dentate gyrus into where lypophilic tracers are to be injected.

Figure 9. The coronal slice injected lypophilic tracer into the apex of the dentate gyrus. The injected site is indicated by arrow.

lypophilic tracer is slowly injected by nanotransjector. The detected apex of the dentate gyrus into where lypophilic tracers are injected is shown in Fig. 8. By using an automated micromanipulation device, the tip of microelectrode is successfully inserted into the apex of dentate gyrus by 6 to 8 steps of movements within 2 to 3 minutes, and 0.5 to 1 μ l of lypophilic tracer is injected into granular cell layer. Fig. 9 shows the coronal slice injected the lypophilic tracer into the apex of the dentate gyrus. The injected site is indicated by arrow. The injected slices are returned into fixative and stocked at room temperature or 4°C under light-protected condition. Morphological properties of neurons in granular cell layer, molecular layer, and hilus is analyzed by conforcal laser scan microscope (Zeiss) more than two months after the injection. The results indicate the usefulness of our automated micromanipulation system in neuroanatomical analysis of brain slice neurons.

5 CONCLUSIONS

We have shown that our visual image processing and automated micromanipulation could be utilized for lypophilic tracer injection into the dentate gyrus of rat brain slice. Local injection of tracers has been done by microscopic visual inspection and manual control of microelectrode. However its general use is limited because sophisticated techniques and much burdens are required to operate micromanipulator. Especially when tracers, chemoatractants or neurotrophic factors must be injected into the areas with exact distance from the implanted neurons or regions to where growth cones of axons or dendrites are introduced, it is difficult to inject them in unfixed, unstained brain slice. Our system may be useful in these experimental studies.

REFERENCES

[1] D.G. Amaral, M.P. Witter, The three-dimensional organization of the hippocampal formation: a review of anatomical data, Neuroscience 31(1989) 571-591.
[2] A. Agmon, L.T. Yang, E.G. Jones, D.K. O'Dowd, Topological precision in the thalamic projection to neonatal mouse barrel cortex, Journal of Neuroscience 15(1995) 549-561.
[3] M.E. Dailey, S.J. Smith, The dynamics of dendritic structure in developing hippocampal slices, Journal of Neuroscience 16(1996) 2983-2994.
[4] T. Fukuda, T. Kosaka, Gap junctions linking the dendritic network of gabaergic interneurons in the hippocampus, Journal of Neuroscience 20(2000) 1519-1528.
[5] P. Godement, J. Vanselow, S. Thanos, F. Bonhoeffer, A study in developing visual system with a new method of staining neurons and their processes in fixed tissue, Development 101(1987) 697-713.
[6] M.G. Honig, R.I. Hume, Fluorescent carbocyanine dyes allow living neurons of identified origin to be studied in long-term cultures, Journal of Cell Biology 103(1986) 171-187.
[7] R. Linke, M. Frotscher, Development of the rat septohippocampal projection: tracing with DiI and electron microscopy of identified growth cones, Journal of Comparative Neurology 332(1993) 69-88.
[8] H. Ymamoto, T. Sano, Visual feedback system for micromanipulation, WISP'99, Budapest, Hungary, (1999) 353-358.

AUTHOR(S): *Junko Sakiyama[1)], Univ. Prof. Dr. Motoi Okamoto[1)], Univ. Prof. Dr. Hideki Yamamoto[2)]*
1) Faculty of Health Sciences, Okayama University Medical School, 2-5-1 Shikatacho, okayama, 700-8558 Japan Phone; 086-235-6883 FAX; 086-222-3717 E-mail; mokamoto@fhs.okayama-u.ac.jp
2) Faculty of Education, Okayama University, Tsushima, Okayama, 700-8530 Japan
Phone; 086-251-7723 FAX; 086-251-7755 E-mail; yamamoto@cc.okayama-u.ac.jp