Video Article

Improving the Application of High Molecular Weight Biotinylated Dextran Amine for Thalamocortical Projection Tracing in the Rat

Dongsheng Xu^{*1}, Jingjing Cui^{*1}, Jia Wang¹, Zhiyun Zhang¹, Chen She¹, Wanzhu Bai¹

¹Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences

*These authors contributed equally

Correspondence to: Wanzhu Bai at wanzhubaisy@hotmail.com

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Abstract

High molecular weight biotinylated dextran amine (BDA) has been used as a highly sensitive neuroanatomical tracer for many decades. Since the quality of its labeling was affected by various factors, here, we provide a refined protocol for the application of high molecular weight BDA for studying optimal neural labeling in the central nervous system. After stereotactic injection of BDA into the ventral posteromedial nucleus (VPM) of the thalamus in the rat through a delicate glass pipette, BDA was stained with fluorescent streptavidin-Alexa (AF) 594 and counterstained with fluorescent Nissl stain AF500/525. On the background of green Nissl staining, the red BDA labeling, including neuronal cell bodies and axonal terminals, was more distinctly demonstrated in the somatosensory cortex. Furthermore, double fluorescent staining for BDA and the calcium-binding protein parvalbumin (PV) was carried out to observe the correlation of BDA labeling and PV-positive interneurons in the cortical target, providing the opportunity to study the local neural circuits and their chemical characteristics. Thus, this refined method is not only suitable for visualizing high quality neural labeling with the high molecular weight BDA through reciprocal neural pathways between the thalamus and cerebral cortex, but also will permit the simultaneous demonstration of other neural markers with fluorescent histochemistry or immunocytochemistry.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55938/>

Introduction

High molecular weight BDA (10,000 molecular weight), a highly sensitive tracer, has been used for tracing neural pathways in the central nervous system for over 20 years¹. Although the use of the BDA is a common neural tract tracing technique, the quality of BDA labeling can be affected in animals by various factors^{1,2,3}. Our recent study indicated that the optimal structure of BDA labeling is not only associated with a proper post-injection survival time, but also correlated with the staining method⁴. Until now, conventional avidin-biotin-peroxidase complex (ABC), streptavidin-fluorescein isothiocyanate, and streptavidin-AF594 staining methods were used for revealing the BDA labeling in previous studies^{2,3,4,5}. In comparison, fluorescent staining for BDA can be easily performed.

In order to extend the application of high molecular weight BDA, a refined protocol was introduced in the present study. Following the injection of BDA into the VPM of the thalamus in the rat brain, BDA labeling was revealed by the regular method of standard ABC staining as well as by double fluorescent staining, which was carried out for observing the correlation of BDA labeling and basic neural elements or interneurons in the cortical target with streptavidin-AF594 and fluorescent Nissl histochemistry or PV-immunocytochemistry, respectively. Through the reciprocal neural pathways between VPM and the primary somatosensory cortex (S1)^{6,7,8}, we focused our observation on BDA labeling in the thalamocortical projected axons and corticothalamic projected cell somas in the S1. By this process, we expected to provide not only a detailed protocol for obtaining the high quality of neural labeling with high molecular weight BDA, but also a refined protocol on the combination of fluorescent BDA labeling and other fluorescent neural markers with histochemistry or immunocytochemistry. This approach is preferable to study the local neural circuits and their chemical characteristics under a confocal laser scanning microscopy.

Protocol

This study was approved by the ethics committee at the China Academy of Chinese Medical Sciences (reference number 20160014). All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, D.C., 1996). Four adult male rats (weight 250-280 g) were used in this study. All animals were housed in a 12 h light/dark cycle with controlled temperature and humidity, and allowed free access to food and water. The instruments and materials used in the present study were showed in **Figure 1**. Before the surgery, all instruments, such as stereotaxic frame and glass pipette, were cleaned using 70% ethanol.

1. Surgical Procedures

1. Determine the coordinate area of interest in VPM using a stereotaxic atlas⁹ (**Figure 2A**).
2. Prepare a 1 μ L micro-syringe equipped with a glass micropipette (with a tip diameter of approximately 10-20 μ m) (**Figure 1D**) and test it with liquid paraffin.
3. Anesthetize the rats with 7% chloral hydrate (0.7 mL/100 g) by intraperitoneal injection.
4. Once deep anesthesia is confirmed with tail pinch and pedal withdrawal reflex, shave the top of the animal's head with an electric razor and scrub the surgical site 3 times with 10% povidone iodine followed by 70% ethanol respectively.
5. Place the rat into the stereotaxic device by placing blunt ear bars into the ears and place the rat's upper incisors into the mouth holder (**Figure 1E**), and then apply ophthalmic ointment on the eyes.
6. Clean the head skin of the surgical site again using 70% ethanol. Use sterile surgical gloves and towels to maintain the surgery under the sterile conditions.
7. Make a sagittal incision in the skin with a scalpel along the sagittal suture (**Figure 1F**).
8. Scrape the muscle and periosteum away from the skull using sterile cotton-tipped applicators throughout the surgery to control bleeding (**Figure 1F**).
9. Using predefined coordinates from an atlas (**Figure 2A**), determine the location (-3.3 mm Bregma point, 2.6 mm right to midline) of craniotomy (**Figure 1G**).
10. Perform a craniotomy using a burr drill with a round-tip bit (#106) (**Figure 1H**), and continue drilling to about a 1 mm depth within a few minutes until reaching the meninges (**Figure 1I**).
11. Excise the dura mater using microforceps to expose the cerebral cortex over the injection site (**Figure 1I**).
12. Change liquid paraffin in the micro-syringe with 10% BDA (10,000 molecular weight, in distilled water) solution (**Figure 1J**).
13. Mount the syringe into the microinjection apparatus and connect with a micro-pump (**Figure 1K**).
NOTE: The volume injected is dependent on the speed of the micro-pump. Here it was adjusted to 30 nL/min (**Figure 1L**).
14. Under a stereomicroscope, insert a glass micropipette manually with the microinjection apparatus into the VPM through the cortical surface of the brain at the depth of 5.8 mm (**Figure 1M**).
15. Pressure-inject a 100 nL of 10% BDA into the VPM over a period of 3 min (35 nL/min) with a micro-pump (**Figure 1L, M**).
16. After injection, keep the pipette in place for an additional 5 min and then withdraw slowly.
17. Suture the wound with sterile thread (**Figure 1N**). Follow your local animal care committee guidelines for pre- and post-surgical analgesia.
18. Place the rat in a warm recovery area until it regains consciousness and is fully recovered.
19. Return the recovered rat back to its cage.

2. Perfusions and Sections

1. After a survival time of typically 10 days, inject the animal with an overdose of 10% urethane (2 mL/100 g) by intraperitoneal injection to induce euthanasia.
2. Perfuse the experimental rats in the hood (**Figure 1O**).
 1. Once the breath stops, using scissors and forceps, open the thoracic cavity of the rat to access the heart. Insert an intravenous catheter into the left ventricle toward the aorta, and then open the right auricle.
 2. First perfuse with 0.9% phosphate-buffered saline (PBS) at physiological temperature (37 °C) about 1-2 min until the blood exiting from the heart is clear, and then continue with 250-300 mL 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4).
3. After the perfusion, incise the head skin and open the skull, and then dissect out the rat brain. Post-fix the dissected brain in 4% paraformaldehyde for 2 h at room temperature (26 °C), then cryoprotect in 30% sucrose in 0.1 M PBS (pH 7.4) for 3 days at 4 °C until the brain is immersed in the solution (**Figure 1P**).
4. Once the brain is immersed in the solution, divide the brain into three blocks in the coronal direction on the brain matrices (**Figure 1Q**). The central block contains the VPM and S1.
5. Cut the central block of the brain at 40 μ m on a freezing stage sliding microtome system in the coronal direction. Collect these sections orderly in a 6-well dish with 0.1 M PBS (pH 7.4) (**Figures 1R, S**).

3. Standard ABC Staining

NOTE: Free floating sections from every third coronal section of the brain were used for visualizing the BDA labeling with standard ABC procedure¹⁰.

1. Rinse the sections in 0.1 M PBS for about 1 min.
2. Incubate the sections in 1% ABC solution in 0.1 M PB (pH 7.4) containing 0.3% Triton X-100 for 1 h at room temperature.
3. Wash the sections three times in 50 mM Tris buffer (pH 7.4).
4. Stain the sections in a solution containing 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.01% H₂O₂ in 50 mM Tris buffer for about 2-5 min at room temperature.
5. Wash the sections three times in 50 mM Tris buffer (pH 7.4).
6. Mount the sections on microscope slides using standard histochemical techniques (**Figure 1T**; see **Supplemental Video File III**, perfusion and sections).
7. Dry the sections in the air overnight at room temperature.
8. Dehydrate the sections briefly in a series of alcohol (50%, 70%, 95%, 100%) solutions. Submerge the slides in each solution for about 15 s. Do not allow the slides to dry out between each step.
9. Clear the sections in xylene three times, about 20 min.
10. Put 2 or 3 drops of balsam on the slices then place coverslips on the sections.

4. Double Fluorescent Staining for BDA and Basic Neural Elements in Cerebral Cortex

NOTE: In contrast, double fluorescent staining was carried out for observing the correlation of BDA labeling and basic neural elements on the adjacent sections to the above used with streptavidin-AF594 and counterstained with fluorescent Nissl stain AF500/525.

1. Rinse the sections in 0.1 M PBS for about 1 min.
2. Incubate the sections in a mixed solution of streptavidin-AF594 (1:500) and AF500/525 green fluorescent Nissl stain (1:1,000) in 0.1 M PBS (pH 7.4) containing 0.3% Triton X-100 for 2 h at room temperature.
3. Wash the sections three times in 0.1 M PB (pH 7.4).
4. Mount the sections on microscope slides using standard histochemical techniques. Dry the sections in the air for about 1 h.
5. Apply coverslips to the fluorescent sections with 50% glycerin in distilled water before observation.

5. Double Fluorescent Staining for BDA and Interneurons in Cerebral Cortex

NOTE: Double fluorescent staining was carried out for observing the correlation of BDA labeling and interneurons on the representative sections in the cortical target with streptavidin-AF594 and PV-immunochemistry.

1. Rinse the representative sections in 0.1 M PBS (pH 7.4) for about 1 min.
2. Incubate the sections in a blocking solution containing 3% normal goat serum and 0.3% Triton X-100 in 0.1 M PBS for 30 min.
3. Transfer the sections into a solution of mouse monoclonal anti-PV IgG (1:1,000) in 0.1 M PBS (pH 7.4) containing 1% normal goat serum and 0.3% Triton X-100 for overnight at 4 °C.
4. On the following day, wash the sections three times in 0.1 M PBS (pH 7.4).
5. Expose the sections to a mixed solution of goat anti-mouse-AF488 secondary antibody (1:500), streptavidin-AF594 (1:500), and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, 1:40,000) in 0.1 M PBS (pH 7.4) containing 1% normal goat serum and 0.3% Triton X-100 for 1 h.
6. Repeat steps 4.3 to 4.5.

6. Observation

1. Take images of the VPM, thalamocortical axons, and corticothalamic neurons.
 1. Observe the fluorescent samples with a confocal imaging system equipped with objectives lenses (4x, NA: 0.13; 10x, NA: 0.40; and 40x, NA: 0.95). Use excitation and emission wavelengths of 405 (blue), 488 (green) and 559 (red) nm.
NOTE: Here, the confocal pinhole is 152 μ m (4x, 10x) and 105 μ m (40x). The spatial resolution of image capture is 1024 $\times$ 1024 pixel (4x, 10x) and 640 $\times$ 640 pixel (40x).
 2. Take twenty images in successive frames of 2 μ m from each section at the thickness of 40 μ m (Z series).
 3. Integrate the images into a single in-focus image with the confocal image processing software system for three-dimensional analyzing as follows: set start focal plane $\rightarrow$ set end focal plane $\rightarrow$ set step size $\rightarrow$ choose depth pattern $\rightarrow$ image capture $\rightarrow$ Z series.
2. Take brightfield images by a light microscope equipped with a digital camera (4x, NA: 0.13; 10x, NA: 0.40; and 40x, NA: 0.95 lenses). Use an exposure time of 500 ms. Use photo editing software to adjust the brightness and contrast of images and to add labels.

Representative Results

Survival of 10 days post injection of BDA into the VPM was sufficient for producing intense neural labeling on the corresponding cortical areas ipsilateral to the injection side (**Figure 2**). Both conventional ABC and fluorescent staining procedures for BDA revealed the similar pattern of neural labeling on the S1, including anterogradely labeled thalamocortical axons and retrogradely labeled corticothalamic neurons (**Figure 2C, D**).

For the anterogradely labeled axons, they were observed from layer 2 to layer 6 with higher density on the layer 4, and the typical type of thalamocortical axons was observed in the barrel area (**Figure 2C, D**). In the higher magnification view, BDA labeling clearly presented on the axonal trunk, branches, collaterals, and small varicosities (**Figure 3A**). Under the labeled thalamocortical axons to be displayed, the retrogradely labeled cortical pyramidal neurons were displayed, and their cell bodies distributed on the layers 5/6, but their apical dendrites extended to layer 2 (**Figure 2C, D**). The BDA labeling not only presented in the neuronal cell bodies and dendrites, but also in dendritic spines, appearing as Golgi-like resolution (**Figure 3B**).

In addition, double fluorescent staining was also performed for observing the correlation of BDA labeling and interneurons in the cortical target with streptavidin-AF594 and PV-immunochemistry. Around the BDA labeling in the cortical target, PV-positive neurons were distributed from layer 2 to layer 6 with high concentration in layer 4 (**Figure 4A**). There were no PV-positive neurons to be labeled with BDA, however, BDA-labeled axonal terminals were found around the surface of PV-positive cell bodies and PV-positive axonal terminals around BDA-labeled cortical pyramidal neurons (**Figure 4B, C**).

Figure 1. Photographs of key surgical steps and main instruments to be used in this experiment.

A: The stereotaxic device. **B:** The dental drill. **C:** Surgical tools (scalpel, microforceps, and *etc.*) **D:** Micro-syringe equipped with a glass micropipette. **E:** Place the rat head into the stereotaxic device. **F:** Clean the surgical site. **G:** Confirm the Bregma point. **H:** Drill the skull. **I:** Expose the cerebral cortex. **J:** Load 10% BDA into the micro-syringe. **K:** Mount the syringe into the apparatus for micro-injection. **L:** Adjust the speed of the micro-pump. **M:** Insert the glass micropipette into the VPM. **N:** Suture the wound with sterile thread. **O:** Perfuse the rat in the hood. **P:** Dissect out the brain. **Q:** Divide the brain into three blocks with the brain matrices. **R:** Cut the brain on a freezing stage sliding microtome system. **S:** Collect brain sections orderly in a 6-well dish. **T:** Mount the sections on microscope slides. [Please click here to view a larger version of this figure.](#)

Figure 2. Injection site and typical neural labeling with high molecular weight BDA in the somatosensory cortex.

A: Injection site was determined on a stereotaxic atlas. **B:** Representative photomicrograph showing the injection site of BDA (red) in the ventral posteromedial nucleus of thalamus (VPM) on the background of green Nissl staining. **C:** Photomicrograph of fluorescent BDA labeling, including thalamocortical axons in layer 4 and corticothalamic neurons in layers 5/6. **D:** Photomicrograph of conventional BDA labeling with a standard avidin-biotin-peroxidase procedure showing the similar pattern of neural labeling to fluorescent BDA labeling. VPL, ventral posterolateral nucleus of the thalamus. [Please click here to view a larger version of this figure.](#)

Figure 3. High quality of neural labeling with high molecular weight BDA on the background of green Nissl staining.

A: Higher magnification view of the anterogradely labeled axonal arbors (red) in detail. **B:** High resolution photographs showing the retrogradely labeled neuronal cell body, dendrites, and dendritic spines (red) in detail. [Please click here to view a larger version of this figure.](#)

Figure 4. The correlation of BDA labeling and calcium-binding protein PV-positive interneurons in the cortical target.

A: The distribution of BDA labeling (red) and PV-positive interneurons (green) in the somatosensory cortex. **B:** Higher magnification view of the distribution of BDA-labeled axons and PV-positive interneurons in layer 4. **C:** High resolution photograph of BDA-labeled cortical pyramidal cell (red) and PV-positive interneurons (green) in layers 5/6. All samples were counterstained with DAPI (blue). [Please click here to view a larger version of this figure.](#)

Supplemental Video Files: The supplemental videos briefly show the surgical procedure, injection of BDA into the VPM, perfusion and sectioning, and results. In the video file named "IV. Representative Result", the three-dimensional confocal laser scanning microscopy and analysis system results are shown. High resolution animation shows the retrogradely labeled neuronal cell body, dendrites, and dendritic spines (red) in detail. [Please click here to download this file.](#)

Discussion

Selecting a proper tracer is a critical step for a successful neural tracing experiment. In the family of BDA, high molecular weight BDA (10,000 molecular weight) was recommended to be preferentially transported through the anterograde neural pathway in contrast to low molecular weight BDA (3,000 molecular weight)^{2,3,11,12,13}. However, many studies also suggested that high molecular weight BDA might also be potentially used for retrograde pathway tracing in parallel with anterograde tracing^{1,2,3}. Through the reciprocal neural pathways between VPM and S1, we provided further evidence to support the idea that high molecular weight BDA is suitable for bidirectional tract tracing⁴. In a similar way in this domain, high molecular weight BDA was also used in the visual and auditory system for examining the reciprocal connections between the dorsal lateral geniculate body and visual cortex as well as the medial geniculate body and auditory cortex^{3,14}.

In the neural tracing experiment, the other important consideration is the choice of staining method for the neural labeling. In most of the previous studies, both the conventional ABC protocol and fluorescent streptavidin staining method have been used for examining BDA labeling and revealed the similar morphological pattern^{1,2,3,4,5}. Here, we found that streptavidin-AF594 was not only a proper choice for BDA labeling, but also suitable for combining with other neural marker, such as fluorescent Nissl and PV-antigen, providing a new opportunity for observing the correlation of BDA labeling and other neural elements. Although the conventional double-staining method for BDA and other neural markers were also frequently carried out with the two-color DAB procedure^{14,15}, it is not suitable for using a three-dimensional analysis system under a confocal laser scanning microscopy. From a technical standpoint, our present study provides a valuable reference to distinctly compare between BDA labeling and other labeling.

The neural tract tracing technique was initially used for revealing neuronal connections between the injection site and its target in the nervous system; however, researchers still were in pursuit of the ideal structure of neural labeling with a proper tracer^{16,17}. In contrast to other tracers, such as horseradish peroxidase and subunit B of cholera toxin, high molecular weight BDA produces the higher quality of neural labeling for quantifying the number of axonal arbors and neuronal dendrites^{16,17}. Although the quantitative analysis of BDA labeling was not carried out in the

present study, the higher quality of neural labeling with BDA provides more opportunities to closely understand the morphological characteristics on labeled axonal arbors and neuronal dendrites.

Similar to the application of many other tracers, a series of important issues should be considered for this experimental procedure, including: the concentration and volume of BDA used for injection, correct site of injection, the tip diameter of the glass pipette, surgical process, optimal survival time, perfusion, section, and observation under a microscope. It is directly associated with the quality of BDA labeling what we expected. In addition to the critical steps mentioned above, there are technical limitations that require attention, such as the distance from the injected site to the labeled target, which is dependent on the model animal species used in the experiment^{3,5,18}. In a word, a successful tracing study depends on every step in the entire experimental procedure.

In the present study, we have provided a refined protocol to demonstrate that the fluorescent BDA staining method is an effective way for obtaining the high quality of neural labeling, which can be simultaneously combined with other neural markers by using fluorescent histochemistry or immunohistochemistry. Comparing the conventional BDA staining with the DAB procedure, we can more easily analyze the fine structure of BDA labeling and distinguish it from other neural elements in the tracing target under a confocal laser scanning microscopy by the present fluorescent approach.

Disclosures

The authors have nothing to disclose.

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References

- Veenman, C.L., Reiner, A., Honig, M.G. Biotinylated dextran amine as an anterograde tracer for single- and double-labeling studies. *J Neurosci Methods*. **41**, 239-254 (1992).
- Reiner, A., Veenman, C.L., Medina, L., Jiao, Y., Del Mar, N., Honig, M.G. Pathway tracing using biotinylated dextran amines. *J Neurosci Methods*. **103**, 23-37 (2000).
- Ling, C., Hendrickson, M.L., Kalil, R.E. Resolving the detailed structure of cortical and thalamic neurons in the adult rat brain with refined biotinylated dextran amine labeling. *PLoS One*. **7**, e45886 (2012).
- Zhang, W.J., et al. Anterograde and retrograde tracing with high molecular weight biotinylated dextran amine through thalamocortical and corticothalamic pathways. *Microsc Res Tech*. **80**, 260-266 (2017).
- Han, X., et al. Biotinylated dextran amine anterograde tracing of the canine corticospinal tract. *Neural Regen Res*. **7**, 805-809 (2012).
- Armstrong-James, M., Callahan, C.A. Thalamo-cortical processing of vibrissal information in the rat. II. spatiotemporal convergence in the thalamic ventroposterior medial nucleus (VPM) and its relevance to generation of receptive fields of S1 cortical "barrel" neurones. *J Comp Neurol*. **303**, 211-224 (1991).
- Armstrong-James, M., Callahan, C.A., Friedman, M.A. Thalamo-cortical processing of vibrissal information in the rat. I. Intracortical origins of surround but not centre-receptive fields of layer IV neurones in the rat S1 barrel field cortex. *J Comp Neurol*. **303**, 193-210 (1991).
- Agmon, A., Yang, L.T., Jones, E.G., O'Dowd, D.K. Topological precision in the thalamic projection to neonatal mouse barrel cortex. *J Neurosci*. **15**, 549-561 (1995).
- Paxinos, G., Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press, San Diego, (1998).
- Davidoff, M., Schulze, W. Standard avidin-biotin-peroxidase complex (ABC) staining combination of the peroxidase anti-peroxidase (PAP)- and avidin-biotin-peroxidase complex (ABC)-techniques: an amplification alternative in immunocytochemical staining. *Histochemistry*. **93**, 531-536 (1990).
- Fritzsche, B. Fast axonal diffusion of 3000 molecular weight dextran amines. *J Neurosci Methods*. **50**, 95-103 (1993).
- Kaneko, T., Saeki, K., Lee, T., Mizuno, N. Improved retrograde axonal transport and subsequent visualization of tetramethylrhodamine (TMR)-dextran amine by means of an acidic injection vehicle and antibodies against TMR. *J Neurosci Methods*. **65**, 157-165 (1996).
- Medina, L., Reiner, A. The efferent projections of the dorsal and ventral pallidal parts of the pigeon basal ganglia, studied with biotinylated dextran amine. *Neuroscience*. **81**, 773-802 (1997).
- DE Venecia, R.K., Smelser, C.B., McMullen, N.T. Parvalbumin is expressed in a reciprocal circuit linking the medial geniculate body and auditory neocortex in the rabbit. *J Comp Neurol*. **400**, 349-362 (1998).
- Ojima, H., Takayanagi, M. Use of two anterograde axon tracers to label distinct cortical neuronal populations located near. *J Neurosci Methods*. **104**, 177-182 (2001).
- Kobbert, C., Apps, R., Bechmann, I., Lanciego, J.L., Mey, J., Thanos, S. Current concepts in neuroanatomical tracing. *Prog Neurobiol*. **62**, 327-351 (2000).
- Vercelli, A., Repici, M., Garbossa, D., Grimaldi, A. Recent techniques for tracing pathways in the central nervous system of developing and adult mammals. *Brain Res Bull*. **51**, 11-28 (2000).
- Liao, C.C., Reed, J.L., Kaas, J.H., Qi, H.X. Intracortical connections are altered after long-standing deprivation of dorsal column inputs in the hand region of area 3b in squirrel monkeys. *J Comp Neurol*. **524**, 1494-1526 (2016).