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Morphometric Analysis in Neurodegenerative Disorders

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Abstract

The study of dendritic length and spine density has become a standard in the analysis of neuronal abnormalities since a considerable number of neurological diseases have their foundation in alterations in these structures. One of the best ways to study possible alterations in neuronal morphometry is the use of Golgi impregnation. Introduced more than a century ago, it is still the standard and state-of-the-art technique for visualization of neuronal architecture. We successfully applied the Golgi method to mouse, rat, monkey and human brain tissues for studying both the normal and abnormal morphology of neurons. We were able to discover subtle morphological alterations in neuronal dendrites and dendritic spines in different brain areas. Although Golgi preparations can be examined by electronic microscopy, we used light microscopy and Neurolucida reconstruction to quantitatively explore the relationship between total dendritic length and spine density in different types of neurons. This review summarizes the methodology used to quantify neuronal abnormalities and discusses the utility of these techniques in different models of neurodegeneration.

Keywords

Golgi impregnation; Neurolucida; dendrites; dendritic spine; neuronal morphometry

INTRODUCTION

In spite of being developed more than 130 years ago by Camillo Golgi (Golgi, 1873), the impregnation method that bears his name continues to be used as the standard for visualization of dendrites and dendritic spines. Successful impregnation of brain tissue with this method allows qualitative and quantitative characterization of neuronal morphology. Its uniqueness lies in the fact that only a small fraction (fewer than 5%) of the neuronal elements are stained (Spacek, 1989). Groups of nerve cells are stained randomly, and many times selected parts appear completely stained while neighboring areas are devoid of staining. Absence of background staining allows visualization of neuronal structures and dendritic trees to be followed even in thick sections. However, since the cell bodies, as well as the processes are filled with silver precipitate, cytoplasmic details cannot be examined.

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One of the fine structures that were first revealed by the Golgi method and caught the eye of Santiago Ramon y Cayal were the dendritic spines. They were first described by as small thorns that projected from the dendrites of cerebellar Purkinje cells (Ramon Y Cayal, 1888). Although originally thought to be an artifact, dendritic spines are today known to represent centers of information processing that are able to control their own protein synthesis and degradation (Haplain et al., 2005; Melendez-Ferro et al., 2009). As principal sites of synaptic input, spines play a key role in connectivity throughout the brain. Dendritic spine distribution and structure is altered in response to subtle variations in their extracellular milieu due to a number of different factors, including synaptic activity, drugs, toxic exposures and diseases (Fiala et al., 2002). In addition to spine pathology, alterations in the total dendritic length, dendritic branching and soma size have been observed in a number of neurodevelopmental, neurodegenerative and psychiatric disorders, such as Fragile X mental retardation, Down syndrome, Alzheimer's disease, Parkinson's disease, Huntington's disease and schizophrenia (Garey et al. 1998, Glantz and Lewis 2000, Irwin, Galvez and Greenough 2000, Kaufmann and Moser 2000, Fiala, Spacek and Harris 2002, Blanpied and Ehlers 2004, Zaja-Milatovic et al. 2005, Stephens et al. 2005, Knobloch and Mansuy 2008). These structural neuronal changes are usually found to correlate with the disease symptoms. Exercise, stress, and hormonal alterations have also been shown to cause alterations in neuron morphology (Eadie, Redila and Christie 2005, Leggio et al. 2005, Fuchs, Flugge and Czech 2006, Stranahan, Khalil and Gould 2007, Alfarez et al. 2009, Chen et al. 2009, Shansky et al. 2009). Therefore, optimal visualization of neuronal architecture allowed by the Golgi method is of primary importance for the studies of altered neuronal activity and associated morphology in different models of brain disorders.

Currently, there are many variations of the original Golgi method (Millhouse, 1981); most rely on a two-step procedure to impregnate and label cell profiles in the central nervous system (CNS) (Angulo et al., 1994). In the first step, tissue specimens are exposed to a chromating solution, containing either potassium chromate and/or potassium dichromate. In the second step, the tissue is exposed to a silver nitrate solution, which creates the formation of silver chromate crystals (Gabbott and Somogyi 1984, Izzo et al 1987, Jones, 1988; Spacek, 1992). Other procedures eliminate the silver nitrate step and instead of adding mercuric chloride directly to the chromating solution, the tissue is exposed to ammonia to darken the resulting mercury-based precipitate (e.g. Golgi-Cox). There are three major variants of the Golgi method, each exhibiting its advantages and disadvantages, depending upon the condition of the tissue and the particular needs of the study. The Rapid Golgi method utilizes osmium, which can increase the number of cells stained, stabilize the membranes of cells allowing complete impregnation (Spacek, 1992) and promote the rate of crystal formation (Millhouse, 1981). This method works well with fresh tissue (Marin-Padilla, 1995), or tissue that has been fixed in formalin for a very short period of time, from a few hours to a few days. The Golgi-Cox method utilizes a mixture of potassium dichromate and mercuric chloride and has been shown to be highly successful in rats (Glaser and Van der Loos, 1981 and Gibb and Kolb, 1998). The Golgi-Cox method has been reported to be the optimal method for ensuring the staining of entire dendritic trees of cortical neurons, but not for impregnation of dendritic spines (Ramnon-Moliner, 1970 and Buell, 1982), or for studying subcortical structures (Riley, 1979). It reportedly gives reliable, progressive, controlled impregnation of neuronal processes, and stains dendrites very darkly (Buell, 1982). However, this method produces a much lighter background than the rapid Golgi technique and is successful in young brains, as well as heavily myelinated adult brain tissue (Millhouse, 1981). The Golgi-Kopsch method uses formaldehyde and glutaraldehyde as replacement for osmium tetroxide (Kopsch, 1896 and Colonnier, 1964). This method is successfully used in numerous studies for tissues that were fixed in formalin for many years (D'Amelio, 1983), as well as for tissues from perfused animals, immediately taken after brain perfusion and extraction (Riley, 1979; Angulo et al., 1996).

In our morphological studies we have used the FD Rapid GolgiStaining Kit, a commercial Golgi-Cox staining system that has been proven to produce good results in animal and postmortem human brain tissues. This method resulted in an adequate number of well impregnated neurons with nicely visible spines. Tracing and quantitative neuronal morphology was performed with the use of the Neurolucida system (MicroBrightField, Williston, VT). Neurolucida is advanced scientific software for performing neuron tracing, morphometry, as well as brain and anatomical mapping. Neurolucida can be used with live images from color video cameras or with stored image sets from confocal microscopes, electron microscopes and scanning tomographic sources. When used in connection with light microscopes, Neurolucida utilizes a computer controlled motorized XYZ stage for integrated navigation through tissue sections. Once the tracing is complete, images can be transferred to NeuroExplorer, a 3D visualization and morphometric analysis program designed for data analysis, permitting determination of total dendritic length and number of spines per neuron. A representative neuronal tracing from our previous study with monkey brain is presented in Figure 1.

The following Basic Protocol is a description of the methodology for impregnation and staining of neuronal tissue and concrete examples of quantitative characterization of neuronal morphology in studies conducted by our Laboratories.

BASIC PROTOCOL

Golgi-Cox Stain - Rapid GolgiStain™ kit (FD kit)

The FD NeuroTechnologies Rapid GolgiStain™ kit (FD kit) is based on the Golgi-Cox method of Ramón-Moliner (1970) and Glaser and Van der Loos (1981). It has been used in multiple animal species, as well as human post-mortem tissue to assess neuronal morphometry in a variety of brain regions. The kit is simple and easy to use with few technical requirements.

Materials

- Fresh or formalin fixed brain tissue sample
- Razor blade (e.g. Surgical Carbon Steel #12 single edge VWR 55411-055)
- Phosphate-buffered (PBS) solution
- Double distilled or Milli-Q water (DIW)
- FD NeuroTechnologies FD Rapid GolgiStain™ Kit, PK-401
- 15 mL plastic conical tubes
- 1% bovine serum Albumin (Sigma A3912)
- Gelatin from porcine skin, Type A (Sigma G2500)
- Superfrost Plus microscope slide (25 × 75 × 1 mm; e.g. VWR 48311-703)
- Histology cassettes
- Tissue-Tek O.C.T. Compound (Sakura Finetek 4583)
- Plastic staining dishes and rack (e.g. Sakura Finetek 4451)
- Ethanol (100% EtOH)
- Xylene (Richard Allan Scientific, Kalamazoo, MI)
- Cytoseal XYL mounting solution (Richard Allan Scientific, Kalamazoo, MI)

Disposable pasture pipette

Coverslips (24 × 50 mm, no. 1 1/2, e.g. VWR 48393-241)

Manual Rotary Microtome for routine sectioning (Leica RM 2125, Bannockburn IL)

Light microscope Olympus BX62 with motorized stage

Neurolucida and NeuroExplorer software (MicroBrightField, Williston, VT)

Tissue Preparation—Clean and rinse all containers with distilled water. Plastic containers are preferred, but glass containers are acceptable. Do not use metal implements when Solutions A and B from the FD Kit are present. Keep containers closed tightly at all times. Protect tissues from light during and after exposure to Solutions A and B. Perform all procedures at room temperature, unless specified.

1. At least 48 hours prior to experiment, equal volumes of Solutions A and B should be combined and left unstirred in the dark. Use at least 5 ml of impregnation solution per cubic cm of tissue.

**WARNING* Solutions A and B contain mercuric chloride, potassium dichromate and potassium chromate and are toxic to the skin and are fatal if swallowed.*

2. Deeply anesthetize experimental animals prior to sacrifice. The brain should be removed from the skull quickly, but damage to the brain should be avoided. Brain should be dissected depending on specific brain regions of interest.

3. Briefly rinse tissue with double distilled or Milli-Q water to remove blood from the surface of the brain.

Impregnation

4. Place tissue into 15 ml conical tubes containing the impregnation solution for 2 weeks at room temperature in the dark.

Only use the top part of the impregnation solution and avoid the precipitate.

5. Replace the impregnation solution after the first 6 hours of immersion or on the next day.

Dispose of the impregnation solution in accordance with the institutional hazardous waste regulations.

6. Transfer the tissue into Solution C and store in the dark at 4°C for at least 48 hours and up to one week.

7. Replace Solution C after the first 24 hours of incubation or on the next day.

8. After one week processing in solution C in the dark at 4°C, transfer the tissue into the new vial and fill with water. Wash the tissue under the running water over night at room temperature.

9. Place the very well rinsed tissue onto histo-cassette, immerse cassette into 70% ethanol and process in automated histology processor.

All submitted tissue should be placed in cassettes and properly labeled with a unique identification number.

Tissue Processing in Automated Tissue Processor—After fixation, post-fixation and preparatory procedures, the tissue is ready to go through the process of dehydration, clearing, infiltration and embedding. Tissue processing and embedding in wax provides a

support matrix for the tissue. Tissue must be completely fixed before processing. Processors have many containers of reagents into which the samples are transferred sequentially (Carousel) and are pumped in or out.

10. Immerse tissues in increasing concentrations of alcohol to remove water from the tissue. After dehydration, samples are cleared in xylene and infiltrated in paraffin with a Thermo Excelsior Processor

Poor dehydration results in poor processing. Too much time in alcohol can cause artifacts such as vacuolization and shrinkage. Incubation in clearing agents for too long can harden tissue and make sectioning difficult.

11. Samples are immersed in molten paraffin wax which when solidified provides a support matrix.

Paraffin blocks sectioning

12. Paraffin blocks are sectioned at 50–80 μm on a Leica RM2255 automatic microtome.

13. Float sections in purified water at 10°C below the melting point of the wax used, and pick them up on charged slides.

14. Drain upright and dry at 37°C for a minimum of 20 minutes.

15. Leave sections in histology oven at 60°C for at least 1 hour (preferably overnight) to improve adhesion.

16. Paraffin blocks are sectioned at 50–80 μm on a Leica RM2255 automatic microtome.

Alternatively, processed tissue can be frozen and prepared as frozen sections with cryostat.

- a. Rapidly freeze tissue block by gently wrapping it in aluminum foil and placing in the cryostat chamber for at least 10 minutes.
- b. Apply tissue embedding compound on the chuck and allow it to solidify. Set the cryostat thickness to about 100 μm and cut through embedding compound until a flat surface large enough to accommodate the tissue is obtained.
- c. Orient the tissue so that the side of least importance is facing the chuck. Place a small amount of tissue embedding compound on the side of the tissue that is least important and rapidly freeze tissue on the flat surface of the chuck. Place a small amount of tissue embedding compound around the edge of the tissue to secure it to the chuck. Allow tissue embedding compound to solidify for about 5 minutes.
- d. Cut 50 to 150 μm thick sections quickly on a cryostat at -22°C . Set the chamber temperature to one degree colder than the specimen head temperature. Set the cryostat temperature the evening before use to allow sufficient time for the chamber to reach the desired temperature.
- e. Fill the dropper with Solution C and place a drop of Solution C at each position on the gelatin-coated microscope slide where each section will be placed. Make sure that the sections are not placed too close to the edge of the slide. This will cause bubbles to form in the mounting media and render the slides unusable.
- f. Transfer each section onto a drop of Solution C using the glass specimen retriever to mount on gelatin-coated microscope slides.
- g. Absorb excess solution using a strip of filter paper or kimwipe. Absorb excess solution so that sections do not fall off of the slides.

- h. Dry sections flat at room temperature overnight in the dark without the use of a heating source or fan.
- i. Dried sections should be processed as soon as possible, but they may be stored for up to one week in a slide box at room temperature. Proceed to step 20.

Deparaffinization and staining procedure

- 17. Deparaffinize sections in xylene, 3 times, 5 minutes each.
- 18. Hydrate sections in 100%, 95%, 70% and 50% ethanol 2 times, 5 minutes each.
- 19. Rinse in distilled water.
- 20. Rinse sections in double distilled or Milli-Q water 2 times, 2 minutes each.
- 21. Place sections in a mixture consisting of 1 part Solution D, 1 part Solution E and 2 parts double distilled or Milli-Q water for 10 minutes.

Solution D 10 ml

Solution E 10 ml

DIW 20 ml

Prepare working solution just before use. It may be used for up to 200 sections (e.g. mouse brain) per 100 ml, depending on the size of sections. Cover the bottle and staining jar containing the working solution to prevent vaporization of the reagent.

- 22. Rinse sections in double distilled or Milli-Q water 2 times, 4 minutes each (distilled water should be renewed frequently).
- 23. Counterstain sections with cresyl violet if desired.

Tissue Dehydration and Coverslipping

- 24. Dehydrate sections in successively higher ethanol concentrations for 4-minutes each, 50%, 75% and 90% ethanol.
- 25. Continue tissue dehydration in 100% ethanol 4 times, 4 minutes each.
- 26. Clear in xylene or Histoclear 3 times, 4 minutes each.
- 27. Take out one slide at a time and drain off excess solution on a paper towel.
- 28. Add a sufficient amount of Cytoseal to cover the slide and carefully place coverslip on slide. Place more Cytoseal around the edge of coverslip to ensure a complete seal is made. Make sure no air bubbles form between the coverslip and slide.
- 29. Dry the slides at room temperature for 24–48 hours or until completely dry. *Slides should be protected from light.*

Stained slides are now ready to be microscopically evaluated using a light Microscope (Olympus BX62) and Neurolucida.

Neuron Reconstruction—It is important to be careful about which neurons are chosen to be traced for morphometric measurements. Knowledge of typical neuron morphology in the brain region of interest is crucial to making accurate measurements of the correct neuronal subtype. Roitman (2002) describes criteria that neurons are required to meet in order to be considered for morphometric analysis. Selection criteria may include complete filing of the cell body, no beading or breaks in staining along the dendritic branches, limited crossing of dendrites from other neurons, a minimum number of dendritic branches originating from the soma and a minimum number of subsequent branch points. Neurons that meet these criteria

should be traced using Neurolucida software. Full reconstructions of the individual neurons is time consuming, but provides the most information about the neurons. Information includes parameters such as soma size, total dendritic length, total spine number, total dendritic spine density, spine density as a function of distance from the soma and spine density as a function of branch order. If prior knowledge exists about damage to specific regions of the neuron in a particular disease state or toxicant treatment, then full reconstructions may not be necessary and neuronal tracings may be restricted to these areas. This should only be done in circumstances where there is ample and consistent data available to support the use of partial reconstructions.

Statistics—Data from at least 5 neurons in a number of sections from at least 4 animals should be obtained. The ultimate animal and neuron number should be determined by the use of power calculations. Statistics should be performed as in standard experimental situations. In the case of two experimental groups, a Students t-test should be utilized and if there are more than two experimental groups ANOVA should be used in combination with appropriate post-hoc tests.

Reagents and Solutions

0.5% Gelatin coated slides—Heat 100 ml water to 55°C and add 0.5 g porcine gelatin type A and stir until completely dissolved. Dip slides 3 times into gelatin and dry in a 95°C oven for at least 15 minutes or until dry. Store slides in slide box or slide rack at room temperature indefinitely.

COMMENTARY

Expected results

We report on the utility of the FD Rapid GolgiStain Kit for the study of neuronal morphometry in animal and human brain tissue. Our studies show that this impregnation technique is suitable for the analysis of parameters of neuronal morphology, such as dendritic length and spine density, both in fresh brain and archived brains fixed in formalin up to several years. We have used the Neurolucida system with NeuroExplorer quantitative analysis, exploring alterations in neuronal morphology in several models of neurodegeneration including innate immunity activation (Milatovic et al., 2003, 2004), excitotoxicity generated by kainic acid (KA) (Zaja-Milatovic et al., 2008), and neurotoxicities associated with manganese (Mn) exposure (Milatovic et al., 2009) and anticholinesterase agents (Milatovic et al., 2009; Gupta et al., 2007).

In the mouse model of activated innate immunity we have evaluated the integrity of pyramidal neurons from CA1 hippocampal area. As detailed in the previous section, we have used Golgi impregnated (FD Rapid GolgiStain Kit) 50 µm thick hippocampal sections from paraffin-embedded blocks. For glial innate immune response we have used a single intracerebroventricular (ICV) injection of lipopolysaccharide (LPS, 5 µg/5 µl), a major component of Gram-negative bacterial cell walls. Data from this study revealed that coinciding with the peak in oxidative damage to cerebral neuronal membranes at 24 h post LPS injection, a significant reduction in the spine density and dendritic length of pyramidal neurons occurred in the CA1 sector of hippocampus. Since LPS itself has no direct toxic effect on neurons, LPS-activated glial innate immune response leads to indirect neuronal oxidative damage and synaptodendritic degeneration exclusively through a CD14-dependent mechanism (Montine et al., 2002; Milatovic et al., 2004). Representative Golgi-impregnated Neurolucida traced pyramidal neurons from CA1 hippocampal area of control and LPS-treated animals are presented in Fig. 2. Interestingly, both the dendritic spine density and dendritic length returned to near basal levels by 72 h post ICV LPS injection, again

coinciding with resolution of oxidative damage in neurons (Montine et al., 2002; Milatovic et al., 2004).

Dendrites were also quantified to the centrifugal method of Sholl (Scholl, 1953), where spine density and length of dendrites arising from the soma are determined in the first- (50 μm), second- (50 μm –100 μm) and third-order segments (100 μm –150 μm) from the center of the soma. Results of this study demonstrated that LPS treatment caused a significant decrease in total dendrite length in the proximal (0–50 μm) and intermediate (51–100 μm) Sholl compartment of CA1 pyramidal neurons (Fig. 3a). Results also revealed that LPS treatment induced a significant decrease in spine density (number of spines per 100 μm of dendrites) in all three (proximal, intermediate and distal) Sholl compartments of CA1 pyramidal neurons (Fig. 3b).

The integrity of hippocampal dendritic system was also evaluated in the model of anticholinesterase neurotoxicity. We have used diisopropylphosphorofluoridate (DFP) as a model compound for organophosphorus (OP) insecticides or nerve agents, and investigated if oxidative/nitrosative damage induced by anticholinesterase exposure is accompanied by dendritic damage in the CA1 sector of hippocampal neurons. Rats treated acutely with DFP (1.25 mg/kg, s.c.) developed onset of toxicity including, seizures and fasciculations within 60 min. At this time point, DFP caused significant increases in biomarkers of cerebral oxidative damage. Furthermore, quantitative neuronal analysis of pyramidal neurons with no breaks in staining along the dendrites from the CA1 area revealed significant reductions in dendritic lengths and spine density (Zaja-Milatovic et al., 2009). Representative images of Golgi impregnated hippocampal sections with their traced pyramidal neurons from control and DFP-exposed animal are presented in Figure 4. Additional morphometric analysis with the Sholl method of concentric circles confirmed an early neuronal damage and showed that anticholinesterase-induced brain hyperactivity targeted the dendritic system with profound dendrite regression of hippocampal neurons (Zaja-Milatovic et al., 2009).

Manganism is associated with alterations in integrity of the dopaminergic innervation of striatal neurons. The medium spiny neurons (MSN) are the target of the dopaminergic innervation of the striatum, comprising more than 90% of striatal neurons (Deutch et al., 2007). MSN have radially projecting dendrites that are densely studded with spines, synapsing with dopamine and glutamate axons and providing the site of integration of several key inputs and outputs of the striatum (Day et al., 2006). Consequently, alterations in dendritic length and dendritic spine number may destabilize the structural basis of synaptic communication and thus compromise MSN function. Therefore, we investigated if dendritic degeneration of MSN was present in the striatum of mice exposed to a single or multiple injections of MnCl_2 .

Representative images of Golgi impregnated (FD Rapid GolgiStain Kit) striatal section with the traced MSN from control animal is presented in Figure 5. NeuroExplorer assisted neuronal morphometry revealed progressive spine degeneration (total number of spines per neuron) of MSN in mice with increase in time and dose of MnCl_2 exposure. Consistent with these effects, Mn^{2+} also induced dose- and time-dependent progressive dendritic damage (total dendritic length per neuron) of MSN (Milatovic et al., 2009).

In these animal models of neuroinflammation, seizures and metal neurotoxicity, we have performed Golgi impregnation (FD Rapid GolgiStain Kit) of fresh brain tissue (immediately after the extraction). However, we have used the same impregnation procedure for the human brain that was fixed in formalin for several years and investigated if dendritic degeneration in neostriatal MSN is associated with Parkinson's disease (PD). We have used brains from the patients diagnosed with PD and age/time in formalin matched controls with

autopsies at the University of Washington performed between 1987 and 2003. The time in fixative for PD cases averaged 9.7 years (median, 12.5 years) and for controls averaged 9.6 years (median, 12.0 years). Portions of fixed putamen (cca 0.3 cm³) were taken from three consecutive tissue slabs: immediately caudal to the anterior commissure (precommissural), at the anterior commissure, and immediately rostral to the anterior commissure (postcommissure). Tissue was sectioned by vibratome in the coronal plane at 80 µm and Golgi impregnation and morphometric analysis performed by observer blinded to diagnosis as previously detailed. Results from that study showed that spine density in all three regions in controls remained essentially constant across regions of putamen in controls but progressively decreased across precommissural to postcommissural regions in patients with PD. Morphometric values from three different regions of putamen from patients with PD or controls are presented in Figure 6. Importantly, morphometric analysis of MSN from these Golgi impregnated formalin fixed brains showed no correlation of time in fixative with dendritic length or spine density in all three regions in control or patients with PD.

The Neurolucida system and morphometric analysis has been also used in our *in vitro* experimental systems. Confocal immunofluorescent images of neurons previously probed with neuronal markers (MAP2, drebrin or spinophilin) were traced with Neurolucida system and dendritic length or spine density per neuron or the segment evaluated by NeuroExplorer in control and amyloid beta (Aβ) exposed primary neuronal cultures. Representative confocal images, neuronal tracings and morphometric evaluation (dendritic length) of the neurons are presented in Figure 7.

Additionally, we have employed the Rapid Golgi method and evaluated impregnation and visualization of dendrites and dendritic spines from fresh mouse brain tissue. Our preliminary results showed successful impregnation of brain tissue with this method allowing quantitative characterization of neuronal morphology. Rapid Golgi impregnated dendritic segment from mouse MSN is presented in Figure 8.

Altogether, we showed the validity of the use of Golgi impregnation technique (FD Rapid GolgiStain Kit) for the study of human and animal brain tissue, as well as studies on neuronal cultures *in vitro*. In addition to minimal background precipitation, this technique produced good clarity of dendrites and dendritic spines in different brain areas. Our preliminary findings with Rapid Golgi technique also showed good neuronal impregnation and sharpness of dendritic spines. Moreover, we showed that the Neurolucida system is effective in evaluating neuronal morphometry both *in vivo* and *in vitro*.

Critical Parameters and Troubleshooting

Tissue sectioning on the microtome or cryostat are critical parameters in the success of either protocol. Trials should be conducted in order to determine the optimal temperature for sectioning on the cryostat and optimal blade advance and amplitude speeds are critical for sectioning tissue on the microtome. The developing stage in the FD Neurotechnologies kit is another critical step, if the sections are left in the developing solution (Solutions D and E) for too long, the tissue will be over stained. When processing is completed tissue should be very well rinsed preferably over night under tap-water to eliminate solution C from the tissue.

Time Considerations

The FD Neurotechnologies kit takes about 3 weeks to complete processing. Using the FD Neurotechnologies kit, it is easy to assess a larger number of animals at one time, because the rate limiting step is sectioning tissue on the microtome/cryostat. The process of sectioning and staining is less time consumptive, and it takes 1–2 days to section, dry and

stain already processes sections. The time investment required to reconstruct the Golgi stained neurons should also be considered, because it is a laborious and time intensive commitment to trace the Golgi stained neurons.

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Figure 1.
Golgi impregnated Neurolucida traced Purkinje cell from the monkey brain.

Figure 2. Neurolucida tracings of representative Golgi-impregnated dorsal hippocampal CA1 neurons from control (A) and icv LPS injected mice. Treatment with LPS induced degeneration of hippocampal dendritic system, decrease in total length of dendrite and spine density of hippocampal pyramidal neurons. Colors indicate the degree of dendritic branching (blue=1°, red=2°, green=3°, yellow=4°, brown=5°).

Figure 3.

Dendritic length (a) and spine density (b) in each Sholl compartment of pyramidal neurons from CA1 hippocampal area of mice following ICV LPS (5 μ g/5 μ l) injections. Brains from rats exposed to LPS were collected 24 h post injections ($n \geq 4$). *One-way ANOVA had $p < 0.001$ with Bonferroni's multiple comparison tests showed significant difference for Sholl compartment ($p < 0.05$) from control (SAL) vs. LPS treatment.

Figure 4.

Photomicrographs of rat hippocampi (2.5X) with pyramidal neurons (10X) from CA1 hippocampal area of rat brains 1 h after saline (control) and DFP (1.25 mg/kg, s.c) injections. Treatment with DFP induced degeneration of hippocampal dendritic system, decrease in total length of dendrite and spine density of hippocampal pyramidal neurons. Tracing and counting were done using a Neurolucida system at 100 x under oil immersion (MicroBrightField, VT). Colors indicate the degree of dendritic branching (yellow=1°, red=2°, purple=3°, green=4°, turquoise=5°, gray=6°).

Figure 5. Photomicrographs of mouse striatal sections with representative tracings of medium spiny neurons (MSN) from mice treated with saline. Tracing and counting are done using a Neurolucida system at 60X or 100 x under oil immersion (MicroBrightField, VT). Colors indicate the degree of dendritic branching (yellow=1°, red=2°, purple=3°, green=4°, turquoise=5°).

Figure 6.

Morphometric values (spine density) from 3 different regions of putamen from patients with PD or controls. 6 to 8 neurons were evaluated in each section and the average value obtained for each region in each individual. These individual averages were then combined to yield a grand average ($\pm$ SEM) for each region in PD patients and controls. Two-way ANOVA for spine density ($F_{42, 2, 1}$) had $P < 0.001$ for 'Control vs. PD', $P < 0.01$ for 'region', and $P < 0.05$ for interaction between these two terms. Bonferroni-corrected post-tests showed that spine density was significantly less in PD patients than controls for commissural and post-commissural regions ($P < 0.001$) but not pre-commissural region ($P > 0.05$).

Figure 7. Representative confocal immunofluorescent images, Neurolucida tracings and NeuroExplorer assisted morphometric evaluation of neurons from control and A β -exposed mixed cerebral and hippocampal neuronal culture. The neurons were probed for MAP2 with mouse anti MAP-2 monoclonal antibody (5 μ g/ml). MAP2 was visualized with cy3-donkey anti-mouse secondary antibody (1:100). Control and A β -exposed neurons were traced out in Neurolucida program followed by quantitation of the dendritic length in NeuroExplorer. A β *-significantly different from control (p<0.01).

Figure 8.
Mouse MSN dendritic segment impregnated by rapid Golgi method.