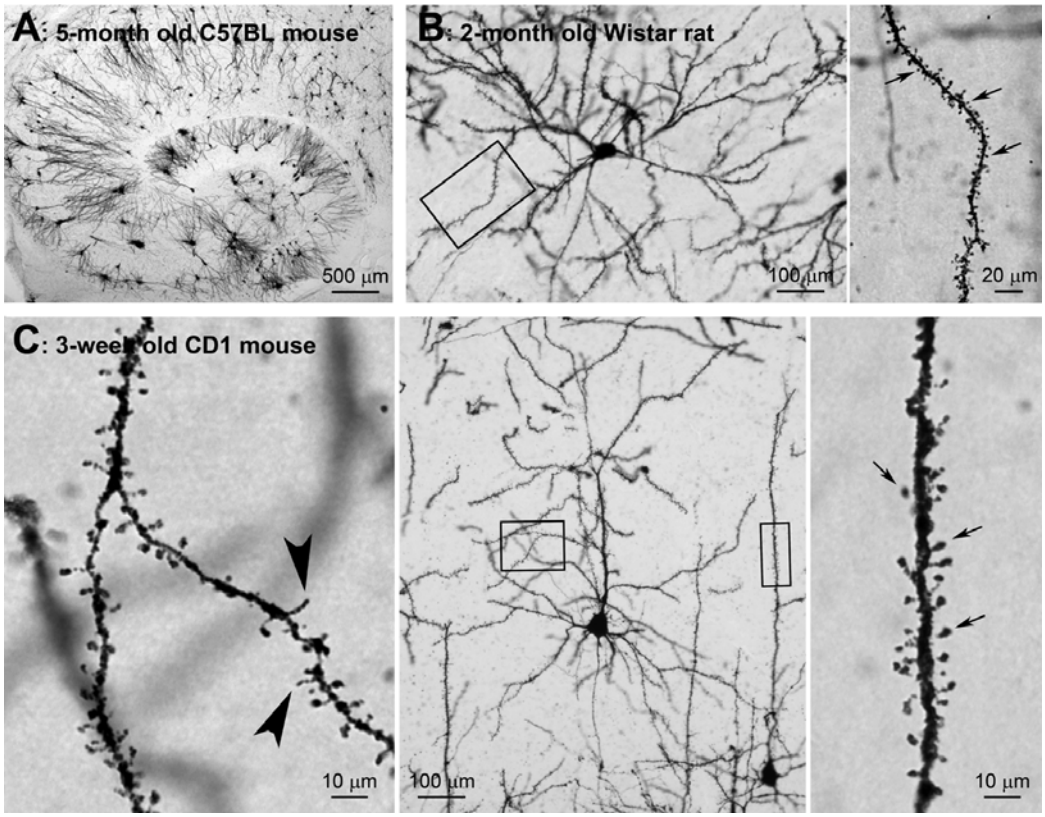
AN ENHANCED GOLGI-COX STAINING SYSTEM FOR DENDRITES/SPINES LABELING

FOR *IN VITRO* RESEARCH USE ONLY

The superGolgi Kit is designed for the labeling of dendritic branches and spines of neurons, based on the principle of Golgi-Cox impregnation (see references below). The Kit has been extensively tested on brain tissues freshly harvested from rats, mice, cats, rabbits, and monkeys, as well as on postmortem brains of humans, yielding a stable and high quality labeling of dendrites and spines (see Figures A-C). Generally, the impregnation of neurons will take 7-14 days depending on the age and size of the tissues (see Table 2). The Kit can be stored in a dark area at room temperature (22 ± 2ºC) for up to 18 months.



Dendritic branches and spines are labeled with the superGolgi Kit. (**A**) Low power (4× objective lens) microscopic photograph shows the Golgi-impregnated neurons in the hippocampus. (**B**) A striatal neuron taken from the posterior caudate. The framed area in the left panel (20×) is magnified in the right to show dendritic spines (arrows, 63×). (**C**) Pyramidal neurons taken from cortex. The framed areas in the middle panel (20×) are magnified to show spines on oblique (left, 100×) and main (right, 100×) branches. Filopodia-like protrusions (arrowheads in left) are frequently observed at this age.

References:

- Ramón-Moliner E: The Golgi-Cox technique. In Nauta WJH and Ebbesson SOE (eds.), Contemporary Methods in Neuroanatomy. pp 32–55, New York: Springer, 1970.
- Glaser ME and Van der Loos H: Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. J Neurosci Methods 1981, 4:117–125.
- Kolb B, Ladowski R, Gibb R, Gorny G. Does dendritic growth underlie recovery from neonatal occipital lesions in rats? Behav Brain Res 1996, 77:125–133.
- Gibb R and Kolb B. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. J Neurosci Methods 1998, 79:1–4.

Warranty: Warranty on the items in the kit is 18 months from the date of purchase.
Return Policy: Bioenno Tech's return policy for this product is 90 days from the date of purchase.
Free Technical Support: Email your questions to contact@bioenno.com

MATERIALS PROVIDED WITH THE KIT:

- Solution **A** (**Impregnation Solution**, 240 ml × 2 QTY) is working solution. It is normal to see precipitate on the bottom, simply use the supernate.

- Reagent **B** [(30 g × 5 QTY) + (30 g × 5 QTY)] is designed for preparation of **two** working buffers. Dilute reagents with distilled or deionized water (dH_2O) before use. The working buffers can be stored at 4ºC for up to 4 weeks.

  ⇨ **Post-impregnation Buffer**: Dilute 30 g of reagent **B** in 90 ml dH_2O
  ⇨ **Collection & Mounting Buffer**: Dilute 30 g of reagent **B** in 500 ml dH_2O

- Solution **C** (**Staining Solution**, 220 ml × 2 QTY). The provided solution **C** must be diluted with dH_2O (**3:5** by volume) immediately before use.

  For example, 18 ml solution **C** + 30 ml dH_2O ⇨ 48 ml of working solution which corresponds to the volume of the staining jar provided. 48 ml of working solution can be used for up to 120 sections (*e.g.*, 12 mouse brain sections per slide and 10 slides total in the provided staining jar).

- Reagent **D** (**Post-staining Buffer**, 90 g × 2 QTY). Dilute reagents with dH_2O before use.

  ⇨ Dilute 90 g of reagent D in 500 ml dH_2O. The working solution can be stored at room temperature for up to 3 months.

  48 ml of working solution can be used for up to 120 sections (*e.g.*, 12 sections × 10 slides in the staining jar as described above).

- Also included: **Shader Paintbrush** (Large and Flat Tip, 1 QTY), for section transfer; **Round Paintbrush** (Small, 1 QTY), for section mounting; **Staining Jar** with cover (2 QTY), for storage, staining, and washing of sections that are mounted on slides.

Materials necessary but NOT included:

Distilled water and/or deionized water (dH_2O);
0.01 M PBS-T (see **Table 1**);
Plastic or glass tubes and bottles;
Plastic forceps;
Histological supplies and equipment: Adhesive microscope slides, Coverslips, Light microscope, Vibratome or micro slicer, Ethanol, Xylene or xylene substitutes, Permount® mounting medium.

Table 1: To prepare 0.01 M PBS-T, first prepare 0.1 M PB (left), and then PBS containing 0.3% Triton X-100 (right).

0.1 M PB, pH 7.4	**1,000 ml**
$NaH_2PO_4 \bullet H_2O$	2.62 g
$Na_2HPO_4 \bullet 7H_2O$	21.73 g
add dH_2O to	1,000 ml
(stir to enhance dissolution)	

0.01 M PBS-T, pH 7.4	**1,000 ml**
NaCl	8.5 g
0.1 M PB (pH 7.4)	100 ml
Triton X-100	3 ml
add dH_2O to	1,000 ml
(stir and heat to 50~55ºC to enhance the dissolution of Triton X-100)	

STORAGE, SAFETY, AND HANDLING PRECAUTIONS:

- Store the kit at room temperature.
- Solutions **A** and **C** in the kit contain highly toxic reagents. Prepare and use them in a fume hood. Collect any waste from solution **A** in a bottle for hazardous waste disposal.
- Wear gloves, appropriate eye and face protection, and suitable protective clothing while handling kit reagents. Wash hands thoroughly after handling.
- Avoid inhalation and contact with skin and eyes while handling. In case of contact, wash immediately and thoroughly with water and seek medical advice if necessary.

PROTOCOL:

- Glass and/or plastic containers with covers are suggested to be used while handling solutions **A** and **C**.
- Keep containers tightly closed at all times. Do not use metal tools to attach solution **A**.
- Protect brains or sections from light while treating them with solutions **A**, **C**, or **D**.
- Do not re-use diluted/working solution, and perform experiments at room temperature for the best results.

1. **Impregnation:** Immerse freshly harvested brain tissues into solution **A**. The volume of solution **A** should be approximately 10 times the volume of the tissue block. For example, ~20 ml of solution **A** would be the correct volume for an adult rat brain (~1cm × 1cm × 2 cm).

   Renew the solution after 1~2 days of immersion, and continue the impregnation at room temperature in the dark (see **Table 2** for suggested total days of impregnation).

   *Suggestions: For optimal impregnation of neurons, it is recommended to perfuse the animal with saline to flush out blood from the tissue. Anesthetize the animal (such as with sodium pentobarbital, 100 mg/kg intraperitoneally), perfuse intracardially or via the ascending aorta with 0.9% saline (*i.e.*, 9 g NaCl in 1,000 ml dH_2O) until the visceral blood volume is flushed out. The perfusion may take 3~5 min. Dissect the brain and remove from the skull with extreme caution. Block the brain with a sharp blade to approximately 1 cm thickness. Rinse briefly in 0.9% saline, followed by immersing in solution **A**.*

   Table 2: Suggested total **Days of Impregnation** under 20-22°C (P: postnatal day; P2 would mean "2-day old").

Animal Age	Size	Impregnation Time
P2 rat	Whole brain together skull	No perfusion, 9 days
P6 rat	Hemisphere	Saline perfused, 7 days
P10 rat	Hemisphere	Saline perfused, 9 days
P24 rat	Hemisphere	Saline perfused, 10 days
P24 rat	Hippocampus block including cortex	Saline perfused, 10 days
3-4 month rat	Hippocampus block including cortex	Saline perfused, 11 days
P6 mouse	Whole brain	No perfusion, 8 days; Saline perfused, 7 days
2-3 month mouse	Whole brain	Saline perfused, 10 days
5 month mouse	Hemisphere	No perfusion, 11-12 days; Saline perfused, 11 days

   Variations in type and size of tissue block may affect the time of impregnation. The ideal time should be obtained by the investigator. Generally, a 2-week impregnation is satisfactory in most cases. If impregnation exceeds 2 weeks, there could be non-specific staining.

2. **Post-impregnation:** When impregnation is ready, rinse the tissue blocks with distilled or deionized water (dH_2O), then transfer them into **B**-prepared **Post-impregnation Buffer** (30 g diluted in 90 ml dH_2O) and store at room temperature in the dark. Renew the solution after one day of immersion, and continue the immersion for another day. After 2 days of post-impregnation, the tissue blocks are ready for sectioning.

The tissue blocks can be stored in Post-impregnation Buffer at 4°C for up to 1 month.

3. **Sectioning:** Sections that are 100-200 µm in thickness can be cut using a vibratome or similar type of microtome. Sections should be collected in **B**-prepared **Collection & Mounting Buffer** (30 g of reagent **B** diluted in 500 ml dH_2O).

   Suggestions: A vibratome or micro slicer is recommended for the sectioning. Set speed and amplitude at Level 4-5 if the scale on the vibratome or micro slicer ranges from 1-10. A thickness of 100-200 µm is suggested for analyses of dendritic branches and spines. For thin section cutting (e.g., 50 µm), immerse the razor blade in xylene for 1-2 min to remove any traces of oil.

4. **Mounting:** With the help of the provided shader paintbrush (large), transfer sections to the **Collection & Mounting Buffer** filled dish (*e.g.*, 95 × 15 mm Petri Dish). Using the round paintbrush (small), mount the sections upon **adhesive** microscope slides. 6~12 sections can be mounted on 1 slide.

   Cover the mounted, wet sections using bibulous paper (such as filter paper), and then press the papers by applying **gentle** pressure to the slides to enhance the adhesion of sections to slides.

   Air-dry sections at room temperature (***long-time exposure to dry air will result in fragmented sections***). The air-dried sections/slides can be stored in a closed and dry staining jar (provided) for up to one day.

   *Suggestions: To prevent any section from falling off the slide during the following **Staining** and **Post-staining**, (1) mount the sections on adhesive microscope slides (such as gelatin-coated slides); (2) wear gloves to avoid any oil or lotion from hands when mounting the sections; (3) press gently the sections upon the slide using bibulous paper; (4) air-dry the sections before washing in PBS-T; and (5) avoid washing of sections/slides in dH_2O.*

5. **Staining:** Wash slides in 0.01 M PBS-T for 20~30 min, and then place the slides in a **diluted** solution **C** (3:5 with dH_2O) for 20 ± 2 min in a closed staining jar (store the jar in a dark area), followed by Post-staining. The ideal staining time should be obtained by the investigator.

   As mentioned in "Materials Provided with the Kit", solution **C** must be diluted with dH_2O (3:5 by volume) immediately before use. For example, 18 ml of solution **C** would be mixed with 30 ml dH_2O to generate 48 ml of working solution, which is the volume of the staining jar provided. 48 ml of working solution can be used for up to 120 sections (*e.g.*, 12 sections per slide × 10 slides). For best results, do not re-use the diluted, working solution.

6. **Post-staining:** Place slides in reagent **D**-prepared **Post-staining Buffer** for 20 ± 2 min in a dark area, and then wash in 0.01 M PBS-T for 5-10 min × 3 times. Air-dry the slides out of direct light, or rinse the slides briefly with dH_2O (1~5 sec) and then air-dry them. Store the slides in a dry and closed jar at room temperature (1 day) or continue to next step "Clean and Cover".

   48 ml of Post-staining Buffer can be used for up to 120 sections (*e.g.*, 12 sections × 10 slides in the staining jar as described in "5. Staining"). For best results, do not re-use the working solution.

   Long-time rinsing/washing in dH_2O will result in sections falling off the slide. Long-time exposure to dry air will result in sections becoming fragmented.

   Optional: After PBS-T washing, sections can be counterstained with cresyl violet, methyl green, or other suitable staining reagent.

7. **Clean and Cover:** Dehydrate sections in 100% ethanol for 5-10 min × 4 times. Clear in xylene or xylene substitute for 5-10 min × 3 times. Cover slip with Permount® mounting medium.

   Store the *super*Golgi-stained sections at room temperature and in a dark area.

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Proven Results
Dendritic branches and spines have been reliably stained and impregnated with the **superGolgi Kit.**

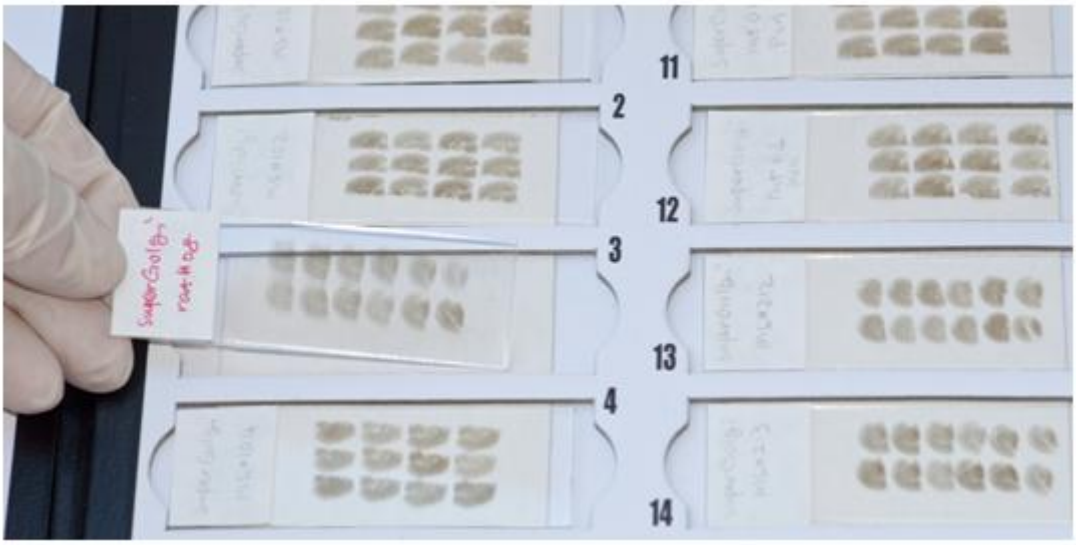

Fig. 1 - Impregnated and Stained Brain Sections Mounted on Adhesive Glass Slides

As shown in Fig. 1, following the **superGolgi Kit** protocol, brain tissues from a rat and mouse were impregnated, sliced, and stained. The stained sections can be stored at room temperature.

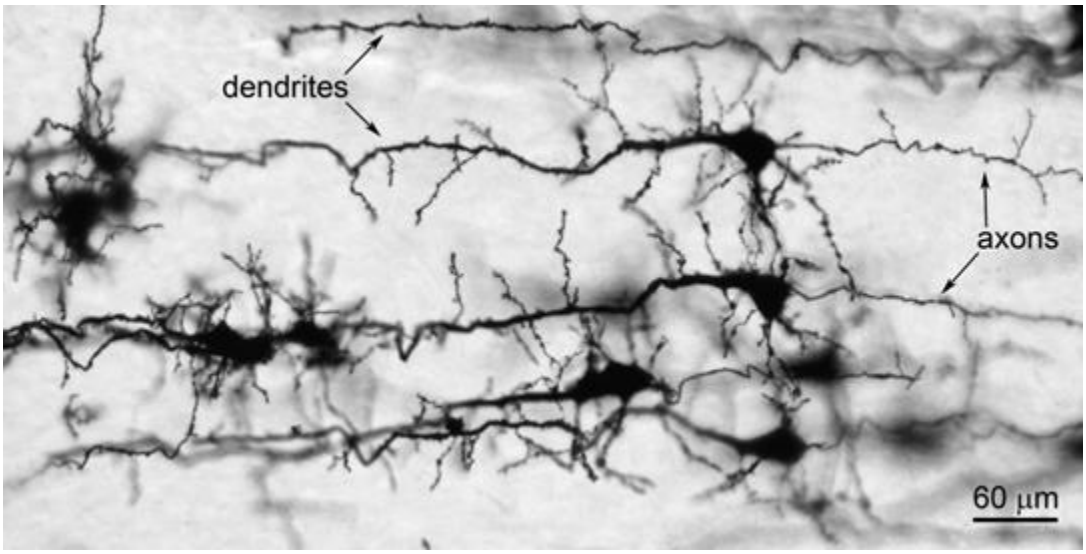

Fig. 2 - Impregnated and Stained Pyramidal Neurons in the Cortex

As shown in Fig. 2, the **superGolgi Kit** was used to impregnate and stain the pyramidal neurons in the neocortex of a postnatal day 2 (P2), C57BL mouse (20x objective lens). Dendrites and axons were well impregnated and stained.

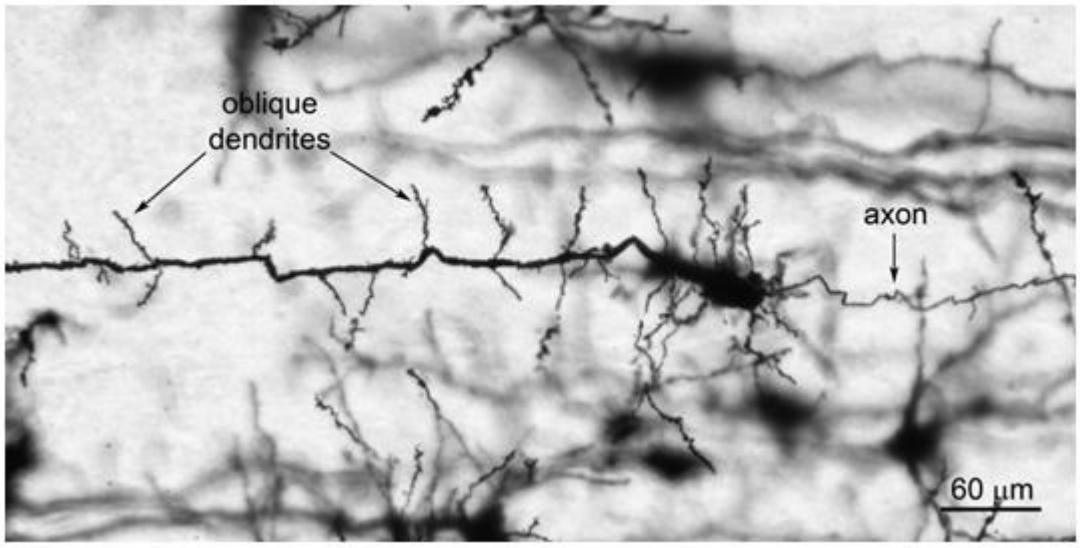

Fig. 3 - Pyramidal Neuron in the Hippocampal CA1 Area

As shown in Fig. 3, the **superGolgi Kit** was used to impregnate and stain the pyramidal neuron in the CA1 area of the hippocampus of a P2 C57BL mouse (20x objective).

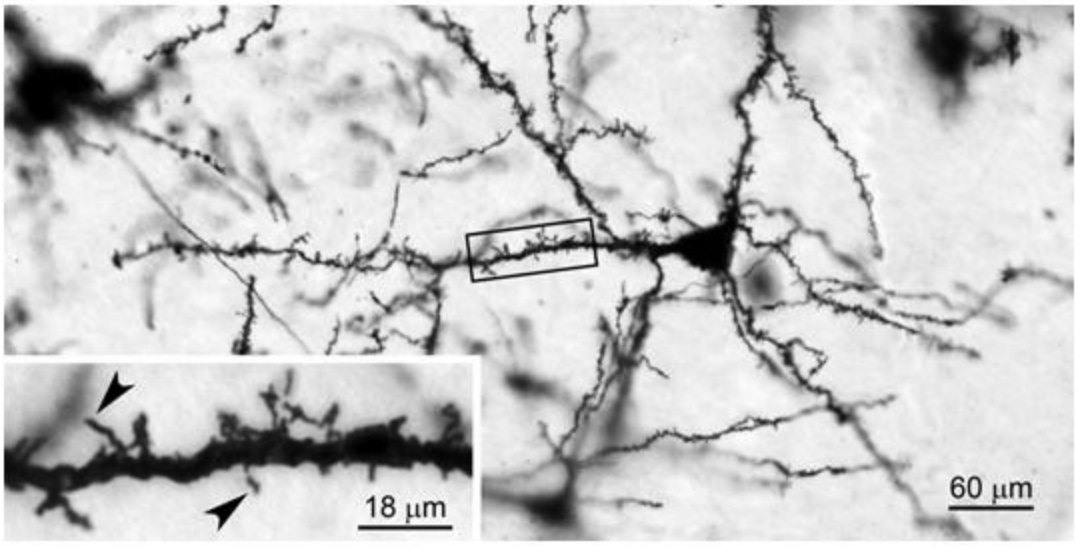

Fig. 4 - Pyramidal Neuron in the Hippocampal CA3 Area

As shown in Fig. 4, the **superGolgi Kit** was used to impregnate and stain the pyramidal neuron in the CA3 area of the hippocampus of a P7 C57BL mouse (20x objective). The boxed area was magnified to show the filopodia-like protrusions (arrowheads), which are the immature dendritic spines.

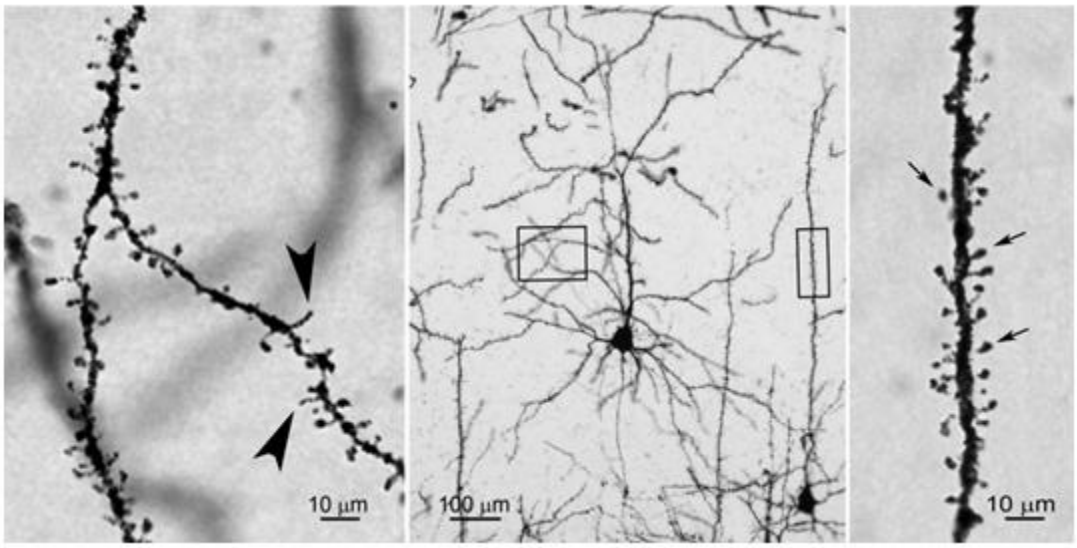

Fig. 5 - Pyramidal Neurons and Labeled Dendritic Protrusions

As shown in Fig. 5, pyramidal neurons were stained using the **superGolgi Kit.** The pyramidal neurons were taken from the frontoparietal cortex (motor area) of a P21 CD1 mouse. The boxed areas in the Middle panel, shown at 20x, were magnified to 100x and presented in the Left and Right panels. On the Left panel, oblique branches are shown. Arrowhead-indicated, filopodia-like protrusions, which are the immature dendritic spines, are often observed at this age. On the Right panel, mature dendritic spines on the main branch are indicated by arrows.

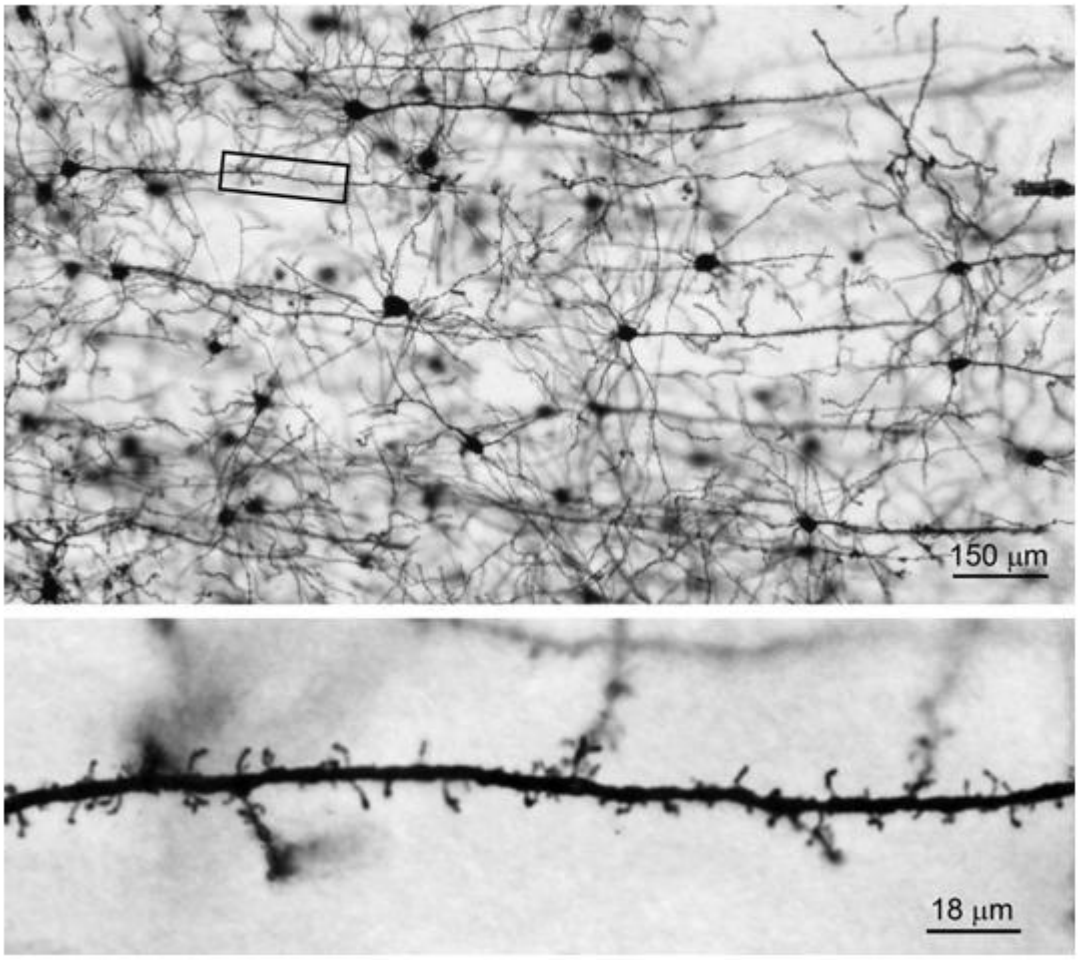

Fig. 6 - Impregnated Cortical Neurons and Labeled Dendritic Protrusions

As shown in Fig. 6, cortical neurons were stained using the **superGolgi Kit.** These neurons were taken from the frontoparietal cortex (somatosensory area) of a P30 C57BL mouse. The boxed area in the Top panel, shown at 10x, was magnified (63x objective) to highlight the dendritic protrusions.

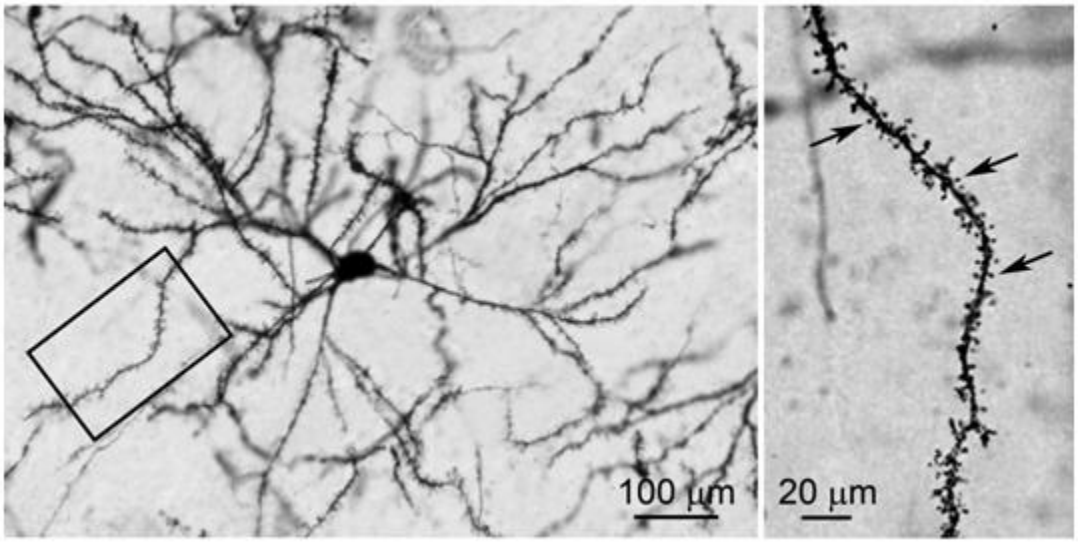

Fig. 7, Left and Right - A Striatal Neuron and Labeled Dendritic Spines (denoted by arrows)

As shown in Fig. 7, the **superGolgi Kit** was used to impregnate and stain the striatal neuron which was taken from the posterior caudate of a 2-month old Wistar rat. (Left Panel: 20x objective; Right Panel: Boxed area was magnified to 63x)

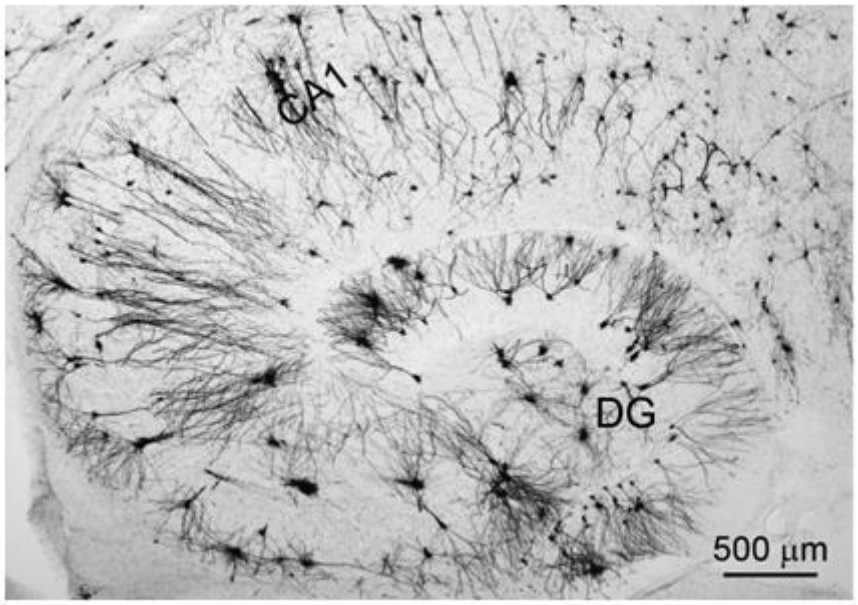

Fig. 8 - Impregnated and Stained Hippocampus

As shown in Fig. 8, the **superGolgi Kit** was used to impregnate and stain the hippocampal neurons of a 5-month old C57BL mouse (4x objective lens). "DG" denotes dentate gyrus.

Importance of Golgi-Cox Impregnation and Staining

Golgi-Cox impregnation and staining allows scientists to clearly visualize the soma, dendrites, and dendritic spines of neurons from the brain tissues of various animals including rats, mice, cats, rabbits, and monkeys, as well as tissues obtained from post-mortem human brains. Understanding the morphology of these cellular structures is critical for research on the effects of various diseases on the brain including Alzheimer's. In addition, understanding these

structures allows scientists to better understand the effects of aging and the effects of man-made toxins and illicit drugs (*e.g.* methamphetamine) on the brain. Furthermore, Golgi-Cox impregnation and staining allows scientists to further understand neuroplasticity and neuroprotection.

Importance of Dendrites of Neurons

Dendrites of neurons make up 95% of the total volume of the neuron. Synapses, which are structures that permit neurons to pass electrical and chemical signals to another cell, are found on dendrites. If there are any changes to the dendrites, this will cause neuron damage. It is important to characterize these dendrites to determine if there is any branching atrophy or spine loss.