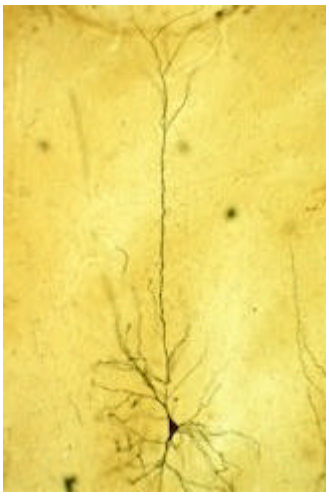
Our Services

Our Services

Using fixed CNS tissue sent to our lab, NeuroStructural Research Laboratories specializes in:

- **Golgi-impregnation staining of neurons**
- **slide preparation, and**
- **in-depth morphometric analysis of both dendritic branching and dendritic spines of the Golgi-impregnated neurons.**



The Golgi impregnation method is a valuable, yet capricious, technique that is used to stain the soma along with entire dendritic arbor and dendritic spines of neurons. (Heavily myelinated axons, due to the lipid content of the sheath, generally resist staining). Although the reasons remain poorly understood, it is widely accepted that the Golgi method stains about 4-6% of the neuronal population and, importantly, that this staining is done at random. Therefore, Golgi staining yields a unique snapshot of neuronal

architecture.

***The importance of Golgi staining is amplified since the dendritic arbor comprises over 95% of the volume of neuron**, **and the dendritic spines are the major loci for synaptic input to the neuron**.*

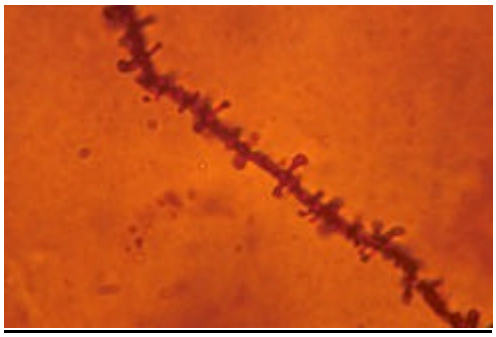
Analysis of these dendritic branching and spines from Golgi-impregnated neurons enables us to generate a unique portrait of the effects of various diseases and treatment strategies on the health and integrity of the neurons and their associated circuitry.

- **Advantage of Golgi Staining: Sensitivity in Assessing Dendritic Branch atrophy, Neuroplasticity, Spine Loss and Formation**

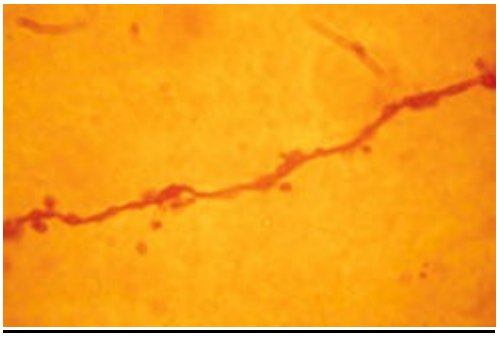
The vast majority of synapses are made directly on dendritic spines that represent the neurostructural loci for transmission of information between neurons. Changes in branching and spines are strongly correlated with cognitive and behavioral parameters. **Synaptic (spine) pathology is a common denominator in virtually all scenarios involving neurodegeneration and cognitive dysfunction regardless of cause** (e.g., genetic, neurotrauma, ischemia, neurotoxicology, etc.)

(Click here to see **Applications** for examples of our technique in various scenarios)

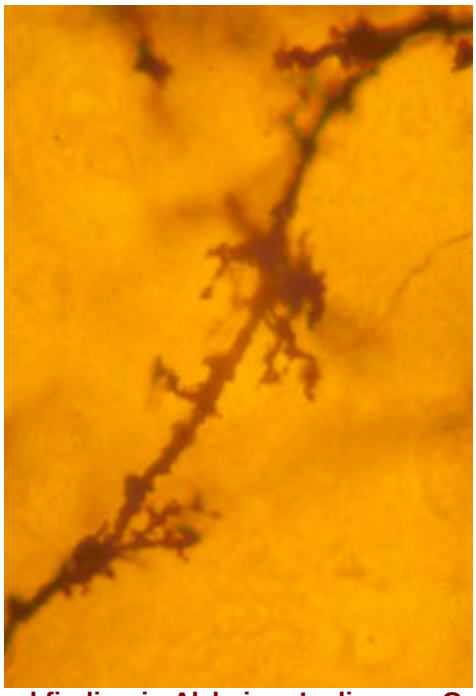
Standard histopathological techniques do not stain dendrites or spines. As such, typical neuronal staining methods may miss early changes in neurodegenerative processes involving dendritic atrophy and spine loss and completely ignore neuroplastic changes involving enhanced dendritic branching and/or new functional or aberrant spine formation.



Normal appearance of dendritic spines on cortical pyramidal cell



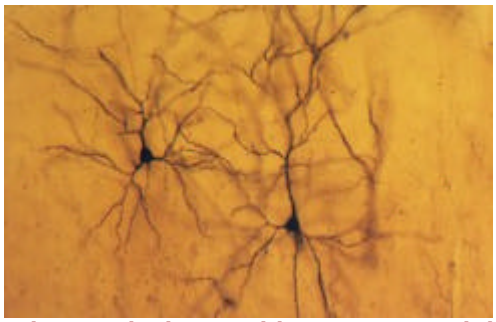
Loss of dendritic spines in a cortical neuron from a patient with Alzheimer's disease. Synaptic dysfunction is a common neurostructural denominator associated with cognitive dysfunction.



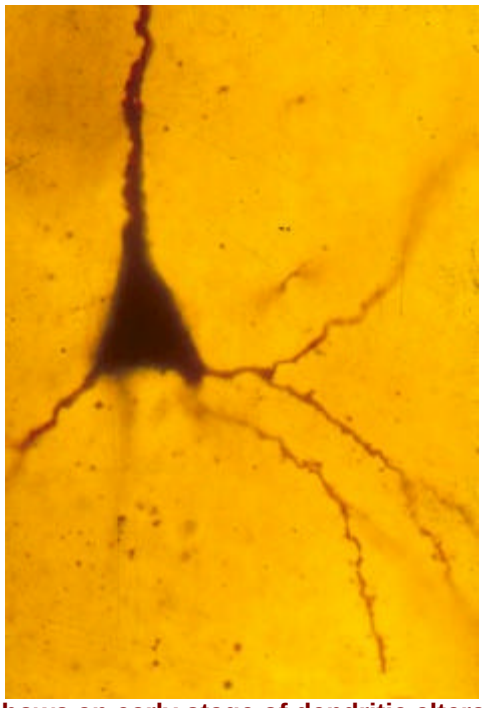
While spine loss is the typical finding in Alzheimer's disease, Golgi staining can also reveal occasionally found aberrant synaptic plasticity .

Stereological assessment of neurons cannot reveal early (e.g., potentially reversible) neurodegenerative effects on dendritic changes or synaptic alterations… nor can it detect neuroplasticity of neuronal branching or spines.

Golgi impregnations -- accompanied by our highly in-depth morphometric evaluations of branching and spines -- are unsurpassed in providing early detection of often-subtle forms of neuronal damage and neurodegeneration (e.g., dendritic atrophy and spine loss). These early changes would be apparent in Golgi impregnations before cell loss could be ascertained.

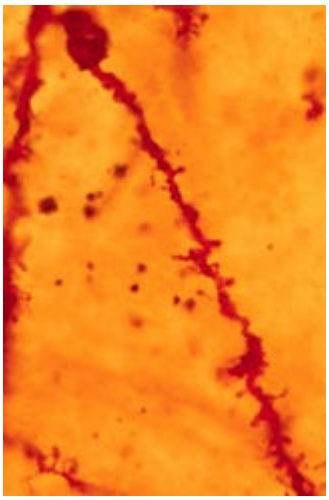


Compared with normal appearing cortical pyramids neurons, Golgi staining shows how we can visualize early (possibly reversible) stages of neuronal atrophy and dendritic spine loss.

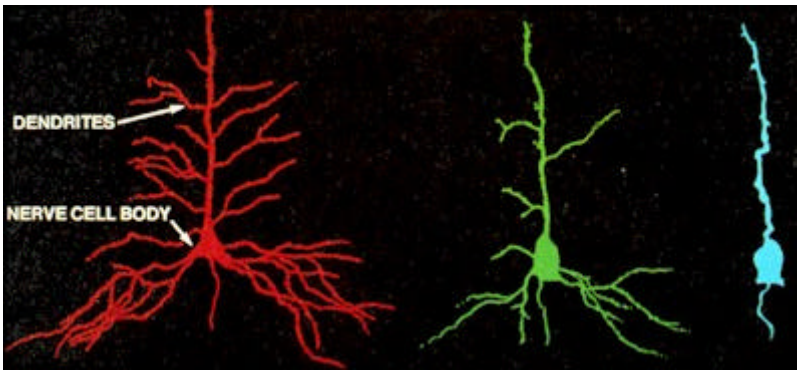


The above photomicrograph shows an early stage of dendritic alterations in a neuron damaged by focal cerebral ischemia.

In the photomicrograph below, we can also clearly see how some of the earlier morphological manifestations of ischemia-related dendritic damage include patchy spine loss, dendritic branch swellings and varicosities.



Using longitudinal type studies (e.g., where animals are sacrificed at various timepoints) we not only can show early and progressive neuronal atrophy, but – if neurons are not too severely damaged – we may also be able to show neuroplasticity and recovery from injury (e.g., regrowth of branching and return to normal spine density). We can also demonstrate how neuroprotective agents can attenuate initial damage or accelerate recovery of the damaged neurons.



As can be seen from the above diagram, we can also demonstrate how neuroprotective agents can attenuate initial damage or accelerate recovery of the damaged neurons.

- **Advantage of Golgi Impregnation: Ease of Tissue Preparation for Staining**

In addition to its sensitivity at assessing dendritic changes, Golgi staining offers another significant advantage over other morphological techniques for assessment of neurons: ***ease of tissue preparation for collaborative studies*.** **(click here to see a list of collaborating institutions and laboratories)**

No special tissue preparation or unusual fixative is necessary for tissue to be sent to our lab for staining and analysis. The standard fixative is 10% neutral buffered formalin. (Other related aldehydes may also be used). Tissue may be immersion fixed or perfused.

Smaller brains (e.g., rats, mice) may be fixed either by immersion fixation or by standard perfusion methods. Other aldehydes (e.g., glutaraldehyde or paraformaldehyde solutions used for or EM preparations) are also acceptable fixatives.

If possible, we prefer to receive tissue that has not been sitting around in fixative for lengthy periods since staining (which can be capricious) may be further compromised. *We may be able to use fixed tissue that is sitting in vials or jars in your lab right now*.

Our **Applications** of this Golgi methodology are diverse and include:

- Brain Development and Models of Mental Retardation
- Brain Aging, Alzheimer's Disease and Neurodegenerative Disorders
- Neurotoxicology
- Genetic Manipulations
- Clinical Neuropathology
- Neurotrauma and Stroke Models

(For more detailed information click here to see **Applications** and our **Abstracts** for examples of these various scenarios)

Just send us your fixed brains or CNS tissue blocks…

NeuroStructural Research Labs will:

- ❑ **Golgi stain the neurons in your tissue and prepare coded slides**
- ❑ **Provide scientifically rigorous and in-depth morphological evaluations comprising:**
  - ♦ **the amount and complexity of dendritic branching**
  - ♦ **length of dendritic branches**
  - ♦ **dendritic spine density and spine configurations**
  - ♦ **soma size**

■ Advantage of Golgi Staining: Permits In-Depth Morphometric Analyses of Dendritic Branching and Spines

While microscopic viewing of Golgi-impregnated tissue provides a unique depiction of the neurons, the greatest benefit is derived from the highly quantitative studies of dendritic branching and spines. The procedures used to generate this information are provided in more detail below.

Operational Procedures and Protocols

- **All the slides are coded. Data is collected using a double blind procedure and no codes are broken until all the data collection has been completed**.
- **We comply with Good Laboratory Practices with respect to tissue handling, generation of the data, and data management.**

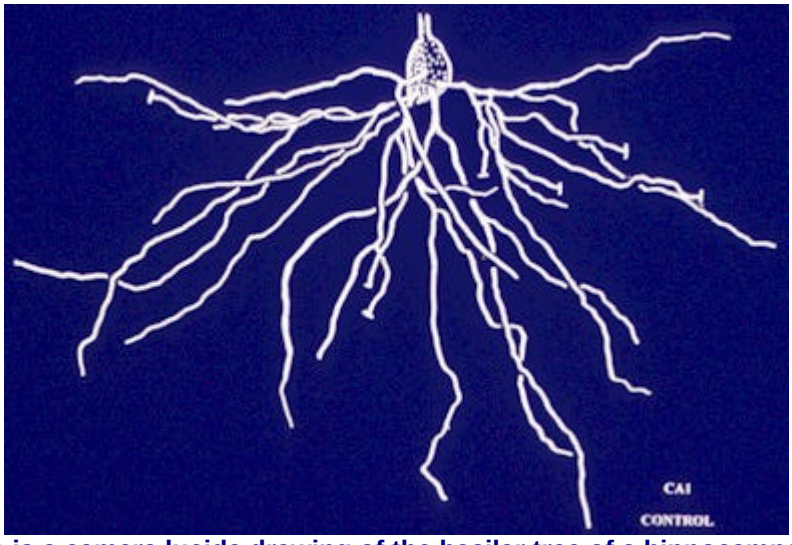
■ How Many Brains Do We Use for a Study?

- Optimally, for statistical purposes, we generally would like to have **5-6 brains (or tissue blocks) per group**.
- From each brain, for a particular cell population from a specific region (e.g., CA1 pyramids of the hippocampus) **we would randomly select 6-7 neurons** from coded slides.
- A typical two-group study would evaluate data derived from approximately 35-40 neurons.
- In some cases, when not as many subjects are available (as, for example, if clinical tissue or monkeys are being studied), we may compensate for the fewer numbers of subjects by increasing the total numbers of neurons from each subject.

- **<u>What are the Basic Steps in a Study and What Kind of Data is Generated</u>?**

Once we have received the fixed brains (or tissue blocks) we carry out all of the following steps necessary for a comprehensive and rigorous scientific study. These steps include:

- Maintaining complete records of all stages of the study
- Dissecting out appropriate regional blocks from the brain for Golgi staining
- Staining the tissue blocks (including preliminary test staining of one or more blocks to optimize impregnation and verify staining schedules)
- Sectioning the stained blocks and preparing slides
- Coding all slides so that the studies are carried out blind (codes are not broken until all the data has been generated and statistical analysis is to be carried out).
- Demarcation of the location of all neurons selected for analysis
- Preparing camera lucida drawings of randomly selected neurons from the selected population for subsequent branching analysis. Converting the camera lucida drawings into quantitative data for statistical comparisons (e.g., sholl analysis)



The above figure is a camera lucida drawing of the basilar tree of a hippocampal CA1 pyramidal neuron)

- Counting dendritic spines from various dendritic regions for spine analysis
- Assessing spine configuration
- Generating measurement of soma size
- Taking appropriate photomicrographs
- Carrying out a complete statistical analysis of all data (with accompanying graphs, photomicrographs, and camera lucida drawings)

- Preparing a final report which includes a discussion and interpretation of the results, with references, and which can serve as a rough draft of a manuscript.

The Advantage of Morphological Information Generated by Golgi-Staining and Neuronal Analysis:

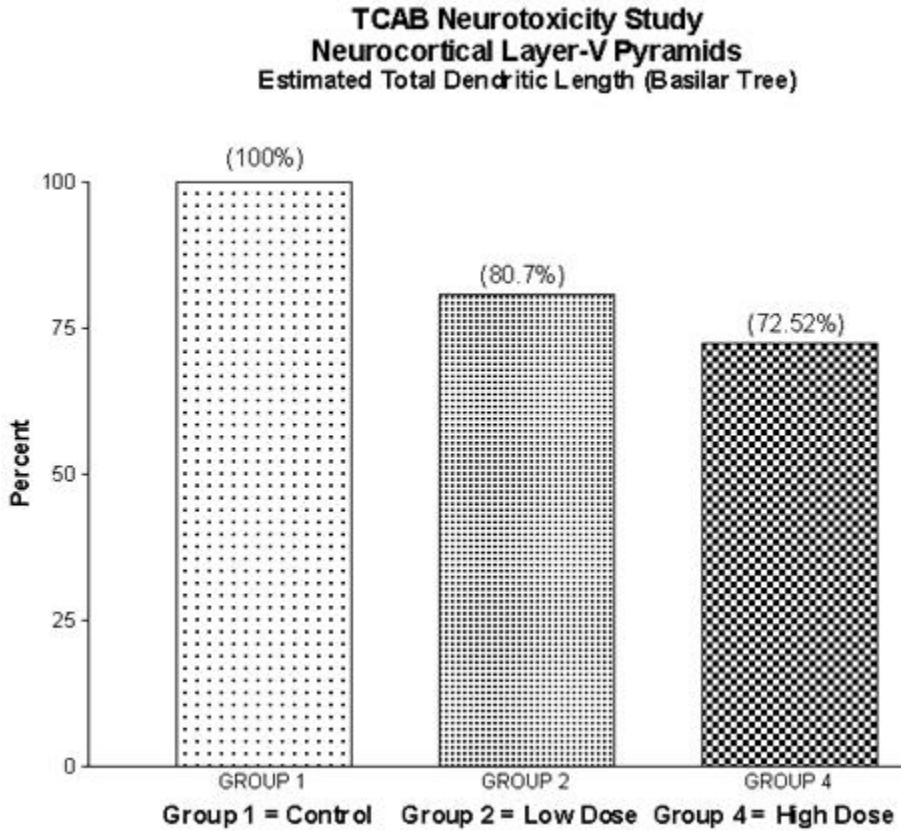
- **Subtle changes in branching and spines reflect the health of the neuronal population as well as the integrity of the neuronal circuitry within the region being evaluated.**

- **Loss of dendritic spines and/or atrophy of the dendritic arbor of cortical or hippocampal neurons is typically associated with impaired learning and memory.**

- **Behavioral alterations and changes in dendritic parameters (dendritic atrophy and/or loss of dendritic spines) are typically found before cell loss per se occurs. Golgi staining represents an exquisitely sensitive means of assessing neurodegenerative and neuroplastic changes.**

- **Analysis of Golgi-stained neurons represents a highly sensitive technique to assess changes in brain circuitry (e.g., amount of dendritic material, complexity of branching, spine density and spine configuration) that ultimately influence learning and memory**

This figure demonstrates how prenatal exposure to increasing dose levels of a neurotoxin, tetrachlorobenzene, (TCAB) resulted in progressively smaller dendritic arbors in cortical neurons in the young adult rat.



Type of Data Generated

For Dendritic Branching

- Sholl Analysis (Method of Concentric Circles): For each of the different groups, this analysis provides a profile of the amount of dendritic material and its distribution.
- Estimated total dendritic length
- Branch Point Analysis: This helps to provide a measure of the complexity of branching of the neurons
- Dendritic Arbor Area

For Dendritic Spines

- spine density
- spine configuration
- estimated total numbers of spines on the dendritic tree
- analysis is generally carried out on internal and terminal tip segment regions

Quantitative assessment of Soma Size

Integration of Data from Branching and Spines

Timetable for the Investigation

The timetable for completion of the study will, of course, depend upon its size and the number of groups. However, in general, a two-group quantitative study of branching and spines will usually take around 3-4 months to complete.

Golgi staining the tissue and preparing slides – including the preliminary test staining (…very important) -- usually takes about six weeks. During the course of the study we will keep you closely informed as to the status of the study and its progress – however, we can't break codes before the study is completed.

Price Structure

The cost of the studies will depend on the size of the study, the nature of the cells to be analyzed, and its complexity. Also considered in the cost is whether only dendritic branching or spines – or both – are to be analyzed. Branching analysis and spine densities can be done separately. You have the option of doing these analyses concurrently or consecutively. Individualized quotes will be promptly provided once we have been contacted.

Once the tissue blocks have been stained and slides prepared, you have the further option of evaluating a second or third neuronal population from the same slides. A typical example might be an initial evaluation of granule cells of the dentate gyrus later followed by a study of CA1 pyramids of the hippocampus, or of layer V pyramids of the overlying fronto-parietal cortex. Since the tissue and all the neurons have already been stained and are located on the same slides there would be a significant savings in analysis of additional neuronal populations.

Please note that if – for any reason – we are unable to stain the tissue or to carry out the study as agreed upon then there is no charge and we will refund any payment already made.

Questions? Need More Information?

Contact Ronald F. Mervis, Ph.D., Chief Scientific Officer, at 813-972-5535 … you can send e-mail to **info@NeuroStructural.org** **….**or you can click on "**Contact Us**" and fill in the reply form and we will make sure that your request for information is promptly answered.

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