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Types of Stains for Neurons

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This brief introduction to the neuroanatomy demo describes the main types of stains used in for revealing different anatomical features of neurons, and how the different types of cochlear nucleus neurons appear under these stains. **Click on thumbnails to see larger pictures**.

- **Nissl stain** (e.g., cresyl violet, thionin, azure) stains nuclei acids (DNA and RNA). This stain is useful for viewing cell sizes and numbers. In animals with cells as large as cats, the pattern of RNA in the cytoplasm, seen as free ribosomes and rough endoplasmic reticulum, is helpful for identifying distinctive neuronal cell classes. Examples include *spherical (***Fig. 1***)* and *globular cells* in the AVCN, whose names reflect their appearance in Nissl stains. *Multipolar (stellate) cells* can usually also be identified. *Octopus* cells have little "Nissl substance" and their shapes must be discerned using other stains.

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- **Reduced silver stains** (e.g. Protargol) stains nuclei and cytoskeletal proteins. This permits visualization of endbulbs in the AVCN (**Fig. 2**), the MNTB, and the ventral portion of the VNLL. Boutons terminaux, which appear as small rings surrounding somata and proximal dendrites are present on some VCN cells (e.g. octopus cells, **Fig. 3**) and on MSO somata and proximal dendrites. Nerve terminals with enough cytoskeleton to be visible using this stain occur in the brainstem auditory nuclei (excluding the DCN) caudal to the inferior colliculus. The functional significance of the cytoskeleton in terminals is not known but it appears to be limited to terminals that have very "secure" synapses.

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- **Golgi impregnations** are not stains, but rather visualization of cells treated in this way results from a small percentage of cells being filled with fine black

crystals. The advantage of this preparation is that it enables visualization of the entire cell body and dendrites. Myelinated axons do not accumulate the salts, although endbulbs, which are not myelinated, may be filled (**Fig. 5**). *Bushy cells* dendrites (**Fig. 4**), which have very few axo-dendritic terminals, have a clearly different appearance from stellate cells' dendrites (**Fig. 6**), which may or may not have many axo-dendritic terminals (Type I and Type II stellate cells). *Type II stellate cells* and *octopus cells* have many axo-dendritic terminals. DCN *pyramidal cells* have two dendritic fields, which extend away from the somata apically and basally. The apical dendrites are covered with spines, which are specializations for expanding the surface area and are the sites of synapses from parallel fibers. *Parallel fibers* are unmyelinated axons of granule cells which course parallel to the DCN surface in the uppermost layer and synapse upon many pyramidal cells' dendrites, which are arrayed in a non-overlapping fashion so that signals from granule cell axons reach successive pyramidal cells at increasing delays. Intermingled with pyramidal cell apical dendrites are *cartwheel cells*, which have spiny recurved dendrites that also are innervated by parallel fibers. Cartwheel cells are inhibitory interneurons that innervate pyramidal cells.

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- **Immunostaining** is a means of visualizing the sites in and on cells where antigens (usually proteins) are situated. The example in this exercise is brainstem auditory nuclei immunostained for the enzyme **glutamate decarboxylase (GAD)**. (It cleaves a carboxyl group from glutamate, which results in production of the inhibitory neurotransmitter GABA, gamma aminobutyric acid.) There is a high correlation of the presence of excitatory terminals and GABAergic terminals upon ventral cochlear nucleus neurons. This demonstration allows one to get a feeling for the density of nerve terminals on these cells by examining GAD immunostained tissue. *Spherical* and *globular cells*, which receive their predominant excitatory inputs from auditory nerve fiber endbulbs, are covered with GAD positive terminals (**Fig. 7**). *Type I stellate cells*, which have few excitatory inputs on somata or dendrites have correspondingly few GAD positive terminals. *Octopus cells*, which receive their excitatory inputs as small terminals from many auditory-nerve fibers, have fewer and smaller GAD positive terminals than spherical, globular, or Type II stellate cells.

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- **Fos immunostaining**

The gene *c-fos* encodes the transcription factor Fos. This gene is part of a family of genes that are quickly induced following sensory stimulation. After the Fos protein has been produced in the cytoplasm it migrates to the nucleus where it forms heterodimeric transcription complexes with other proteins and eventually regulates the transcription of other genes. *c-Fos* expression can be detected by immunostaining (i.e. antibodies against the protein) (**Fig. 8**) or by *in situ* hybridization (i.e. detection of mRNA).

Acoustic stimulation leads to the expression of the gene *c-fos* in auditory neurons in the dorsal cochlear nucleus, ventral cochlear nucleus, superior olivary complex, nuclei of the lateral lemniscus, parts of the medial geniculate nucleus, inferior colliculus, and auditory cortex. Stimulation of animals with pure tones leads to Fos production in restricted tonotopic bands/clusters in most nuclei, hence Fos labeling has been used to study tonotopy without the need for electrophysiology.

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Fig. 8: Fos immunostaining of neurons in the dorsal cochlear (brown reaction product) with Nissl counterstaining (blue). The stimulus was a 25 kHz tone burst, 85 dB SPL for 2 hours. Notice that some cells have darker labeling than others.