Fluorescence of classical microscopic dyes in neural tissue sections of snails

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Fluorochromes are extensively used in conjugates for visualization of specific molecular targets in tissue sections. Fluorescence visualization is favored due to the excellent signal/background ratio and the enhanced effectiveness in multiple labeling techniques. In classical histochemistry, certain fluorochromes also have been introduced to substitute histochemical dyes, which are routinely used in pathology. In our present study the goal was to characterize the extraneuronal environment, including extracellular matrix (ECM) molecules and glia cells in the developing nervous system of snails (*Helix pomatia*, *Lymnaea stagnalis*), which are traditional experimental objects in neurobiologists. ECM and glia system have a crucial role in the organization of developing neurons and their connections, through which they exert a major influence on synapse formation. Applying several types of microscopic dyes on the snail nervous tissue, we found that they can be classified according to their capability of emitting fluorescence light. Metal salts, safranin, thionin, methylen blue did not emit fluorescent light, whereas triphenyl methan based dyes, such as pararosanilin (basic fuchsin) and Vector Red substrate, made the same structures fluoresce, which were also labeled at bright field illumination. The third group of dyes contains toluidin blue, cresyl violet and alcian blue, which labeled different structures at UV or bright field illumination, depending on filter combinations and pH value. pH conditions as determining factor was investigated in detail. It was observed that alcian blue, which labels sulphated proteoglycans with turquoise color under bright field illumination at pH 2.0, also stained neuronal perikarya with red fluorescence at Ex/Em 460/650 filter combination. Toluidine blue labeled DNA with light blue color in the nuclei, RNA with deep blue in the nucleoli and cytoplasm of neurons and glia cells at pH 4.0 under bright field illumination, whereas it labeled the perikaryonal net, the cytoplasm of glia cells and the extracellular matrix of the neuropil (axo-dendritic network) with red fluorescence at the Ex/Em 460/650 filter combinations. An obvious, although not precise, explanation of this phenomenon may be that these dyes are bound to their target in a way, which could only be visualized by UV but not bright field excitation. This staining characteristic of toluidine blue could be used to follow glia cells and neuropil development.

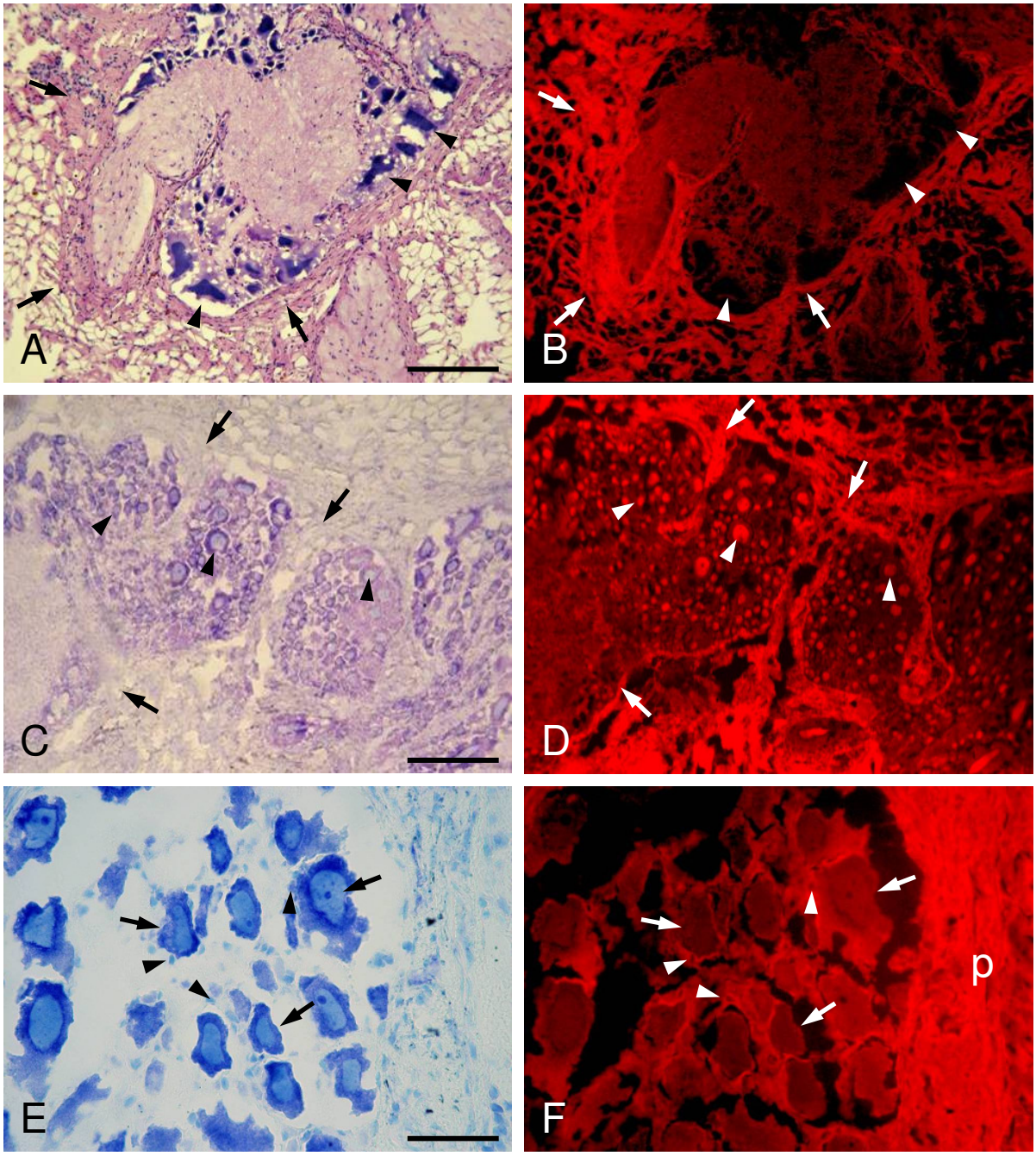

Figure. Histological structures labeled by different microscopic dyes in the same sections (A-B, C-D, E-F) of the snail brain viewed at bright (A, C, E) and fluorescence (B, D, F) illuminations. A-B. Periodic acid-fuchsin stained periganglionic connective tissue (arrows). Neural perikarya visualized by alum-hematoxyline (arrowheads). C. Neural nuclei (arrowheads) stained with cresyl-violet in blue (orthochromatic color), and cytoplasms exhibiting in violet (metachromatic color). pH 5.0. D. Red fluorescence of cresyl violet staining in the nuclei and in the periganglionic ring (arrows). E-F. Toluidine blue labeled blue neural (arrows) and glial (arrowheads) perikarya in E, and red fluorescence in the cytoplasm of glia cells and in the periganglionic connective tissue (p).