

***LEICA VT 1000 E / M***  
***Vibrating Blade Microtome***

**Localization of Nerve Cell Antigens  
in Rat Brain**

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Immunohistochemistry is an important research method to study the central nervous system (CNS). During the last three decades, different immunodetection systems have been developed. CNS antigens can be detected by isotopic, enzymatic and fluorescence systems. Although these methods can be used on brain sections obtained with a cryostat, more accurate localization and superior morphological detail requires the use of non-frozen tissue. Vibrating blade microtomes have been successfully used in such situations. These microtomes use a knife that cuts in two perpendicular directions and the tissue is permanently immersed in a physiological buffer, cooled with ice, to ensure optimum preservation. Sections prepared using a vibrating blade microtome demonstrate excellent preservation of tissue antigenicity and are ideal for morphological studies.

The immunohistochemical localization of neuronal markers has become an important method to study morphological characteristics, pathological states or biochemical changes in different nerve cells of brain. In these studies, good preservation of tissue antigenicity and good penetrability of antibodies are important. The aim of the present work was to localize different CNS markers using the LEICA VT 1000 E vibrating blade microtome. We studied three neuronal markers (Syntaxin, Calbindin D and Heat Shock Protein70) and one astrocytic marker (Glial Fibrillary Acidic Protein).



### Antibodies and reagents

Antibodies raised against different CNS markers (anti-GFAP, anti-MAP2, anti-Syntaxin, anti-Calbindin D, anti-HSP70), secondary antibodies (anti-mouse IgG, anti-rabbit IgG, anti-mouse IgG-FITC, anti-mouse IgG-TRITC, rabbit PAP complex, monoclonal PAP complex), normal serum and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemie GmbH (Germany). All other reagents including Entellan® were analytical grade from E. Merck (Germany).

### Tissue

Sprague-Dawley rats (180-230 g) were perfused with physiological saline (0.85% NaCl) followed by 4 % paraformaldehyde in phosphate buffer saline (PBS). Brains were rapidly dissected and postfixed for 1 hour at room temperature. Tissues were washed in PBS, 4°C overnight. Appropriate pieces of brain tissue were glued onto a specimen plate with Roticoll®, a cyanoacrylate based glue. Tissue sections were obtained with the LEICA VT 1000 E vibrating blade microtome, provided with a buffer tray cooled with crushed ice, to maintain physiological conditions and prevent tissue from drying during sectioning. Sections, 30 µm thick, were obtained using a frequency setting between 8 and 9, and speed <1 on the scale, and processed for immunohistochemistry as follows.

### Immunohistochemistry

Brain sections from different areas were treated for 15 min. at 4°C with methanol/H<sub>2</sub>O<sub>2</sub> (25:100). Following washing in PBS (3 x 10 min.) sections were incubated for 1 hour at room temperature with a blocking mixture containing 5% normal goat serum (NGS) and 3% BSA in Tris buffer saline (TBS). Sections were incubated overnight at 4°C with different sera against several CNS markers diluted in TBS / 5% NGS / 3% BSA. Afterwards, they were washed (3 x 15 min., room temperature) in TBS/ 0.5% BSA and processed differently depending on the detection system. Antisera were diluted as follows: anti-Syntaxin (1/1000), anti-Calbindin-D (1/300), anti-MAP2 (1/400), anti-HSP-70 (1/350) and anti-GFAP (1/80).

### PAP detection system

Brain sections were incubated with goat anti-mouse IgG serum (1/160) - when primary antibodies were monoclonal - or with goat anti-rabbit IgG (1/80) - when primary antibodies were raised in rabbits. After washing in TBS, tissue sections were incubated with PAP complex raised in rabbits or with monoclonal PAP complex. Peroxidase reaction was developed by incubation in TBS containing DAB substrate (0.6 mg/ml) and H<sub>2</sub>O<sub>2</sub> (0.03%). Sections were washed, dehydrated and mounted with Entellan®.

## General discussion

The immunohistochemical localization of the different neuronal and glial cell markers studied here agrees with previous reports. Using sections of fixed brain obtained with the LEICA VT 1000 E vibrating blade microtome, we were able to detect presynaptic (Syntaxin) (Fig. 1) as well as postsynaptic (Calbindin D, HSP70, MAP2) neuronal markers with excellent anatomical resolution. Antiserum raised against HSP, clearly detects this protein in its cytoplasmic localization in normal brain (Fig.11). Calbindin D- and MAP2-positive neurons can be clearly observed in hippocampal, cortical or cerebellar sections (Fig. 6). Note the detection of Calbindin D-positive axons in cerebellum (Fig. 5) and the morphological resolution of axons in Calbindin D- (Fig. 2, 3, 4) and MAP2-positive cells (Fig. 9, 10) in hippocampus and cortex. In these sections anti-GFAP antibodies revealed thin astrocytic processes in the brain cellular matrix and in tight contact with blood vessels (Fig. 7, 8).

Sections used for this study clearly demonstrate absence of cross striations, tissue shrinkage and artifactual staining. Such artifacts are common observations in sections obtained with vibrating blade microtomes. Immunofluorescence detection of CNS antigens in sections obtained with the LEICA VT 1000 E also demonstrate good preservation of tissue morphology and antigenicity (see Fig.12) and therefore their suitability for this type of immunodetection systems. Sections as thin as 10 – 15  $\mu\text{m}$  can be prepared using the LEICA VT 1000 E vibrating blade microtome, these sections, devoid of artifactual staining, demonstrate excellent cellular detail. The technological advances incorporated in the LEICA VT 1000 E allow high quality sections to be prepared for detection of CNS antigens using a large variety of immunodetection systems.

## Light microscopical analysis

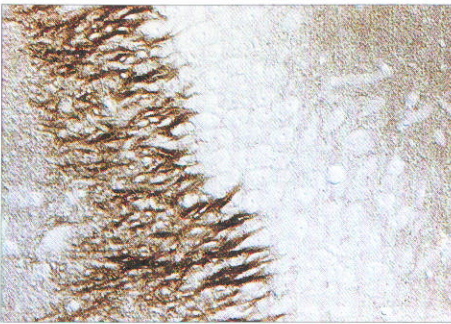


Figure 1: CA3 field of rat hippocampus. Syntaxin positive axon terminals over pyramidal cells. 40  $\mu\text{m}$  section. 400x.

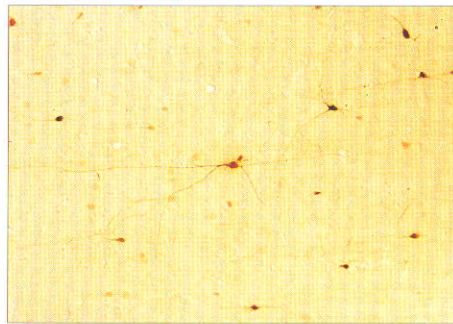


Figure 2: Calbindin D positive neurons in rat brain cortex. 30  $\mu\text{m}$  section. 200x.

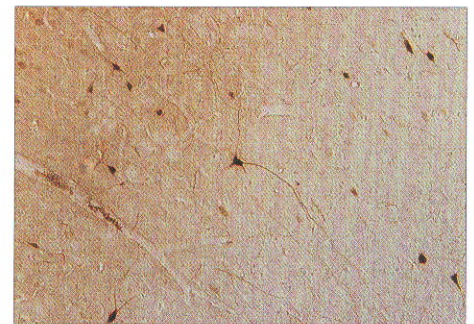


Figure 3: Calbindin D positive neurons in rat brain cortex. 30  $\mu\text{m}$  section. 200x.

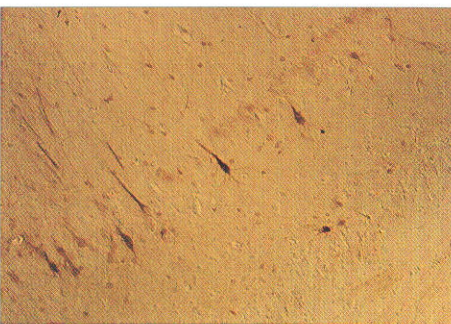


Figure 4: Calbindin D positive interneurons in CA1 field of rat hippocampus. 30  $\mu\text{m}$  section. 200x.

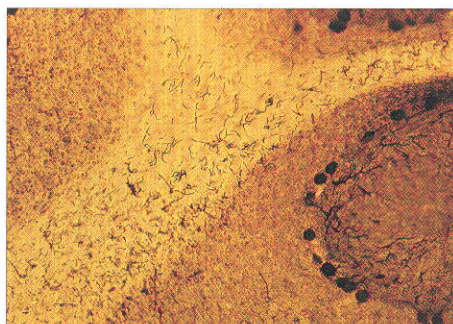


Figure 5: Calbindin D positive Purkinje cells in rat cerebellum. Note the dendritic tree in the molecular layer and the axons running in the white matter. 30  $\mu\text{m}$  section. 200x.

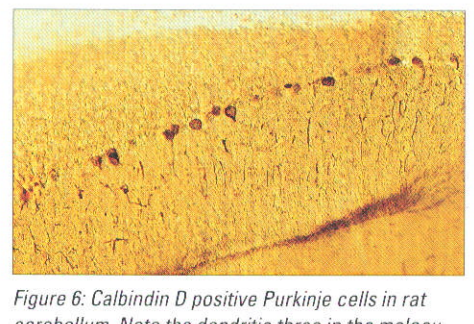


Figure 6: Calbindin D positive Purkinje cells in rat cerebellum. Note the dendritic tree in the molecular layer. 30  $\mu\text{m}$  section. 200x.

## Light microscopical analysis

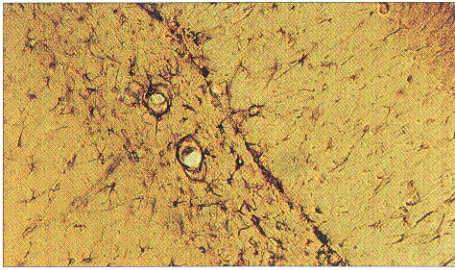


Figure 7: GFAP positive astrocytes in rat hippocampus. Note the astrocyte processes surrounding the blood vessel. 30 µm section. 200x.

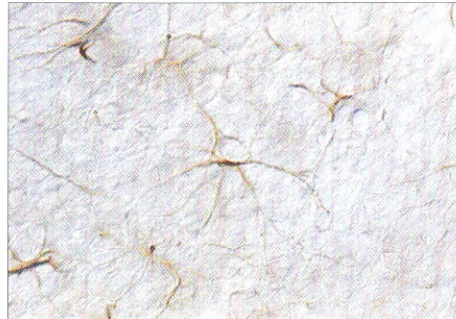


Figure 8: GFAP positive astrocytes in the granular layer of rat cerebellum. 40 µm section. 1000x.

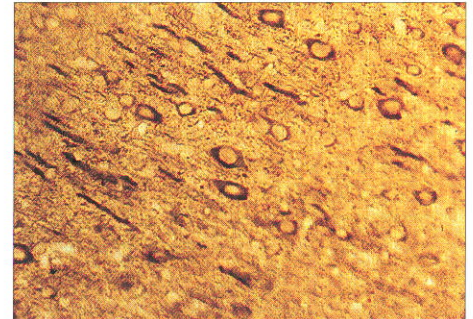


Figure 9: MAP2 positive neurons in rat brain cortex. 30 µm section. 400x.

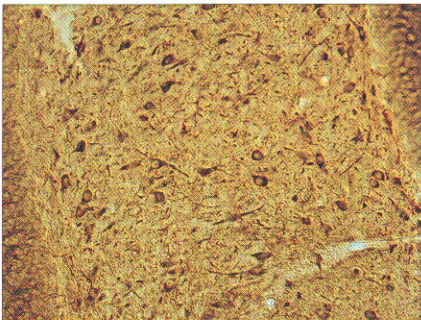


Figure 10: MAP2 positive interneurons in hilus of rat hippocampus. 30 µm section. 200x.

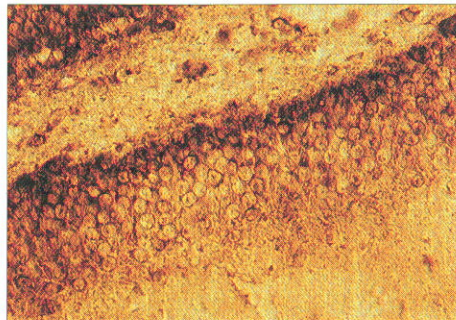


Figure 11: Granule cells of Dentate Gyrus constitutively expressing HSP70 protein. 30 µm section. 400x.

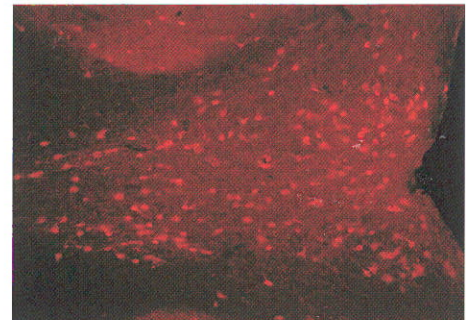


Figure 12: Labeling of cholinergic septal neurons in rat basal forebrain by using a polyclonal antiserum against choline acetyltransferase (ChAT). Slide prepared by Andreas Schober PhD, Department of Anatomy and Cell Biology II, University of Heidelberg, Germany.

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