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Microwave-Assisted MAP-2 Immunoreactivity in Formalin-Fixed, Paraffin-Embedded Guinea Pig Brain.

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Microtubule-associated protein 2 (MAP-2) is the most abundant neuron-specific cytoskeletal protein in the brain, localized specifically in cell bodies and dendrites. The majority of studies have used frozen free-floating sections for detecting MAP-2 for light microscopy (Ballough et al., 1995; Kanayama et al., 1997). The use of frozen brain samples has inherent practical problems associated with storage and freezing artifacts on morphology. Microwave-assisted immunohistochemistry is increasingly being used in diagnostic neuropathology to investigate the expression of neuronal and nonneuronal proteins in formalin-fixed, paraffin-embedded brain tissues. To the authors' knowledge, no reports exist on immunohistochemical staining of MAP-2 in routinely formalin-fixed paraffin brain sections of the guinea pig (Crl: (HA)BR).

The present study tested four antibodies in conjunction with various antigen retrieval (AR) solutions of different pH to examine the efficacy of microwave pretreatment on MAP-2 immunoreactivity in paraffin-embedded sections of formalin-fixed guinea pig brain. Formalin-fixed, paraffin-embedded brain samples sectioned at 5μm and mounted on positively charged slides were dewaxed, hydrated to distilled H_2O, and incubated in 5% hydrogen peroxide at room temperature for 20 min. Serial brain sections were then pretreated two times (5 min each time) in a microwave (Pelco 3440 Max, 800watts) in a 10mM citric acid solution, pH 6.0. After cooling in the same solution at room temperature for 20 min, immunohistochemical localization of MAP-2 was performed using the ABC method of Hsu et al. (1981). Briefly, brain sections were sequentially incubated in primary antibody (1:100), secondary antibody (1:200) and ABC solution (Kan et al., 2001). All brain sections were counterstained with cresyl violet acetate to visualize desired brain areas. To avoid inconsistency of immunostaining specificity associated with tissue handling and staining, brain sections were processed simultaneously.

Microwave-pretreated brain sections exhibited robust MAP-2 immunoreactivity in all brain regions examined. No immunoreactivity of MAP-2 was observed in negative control sections (primary antibody omitted). MAP-2 antibody Ab-3 (Clone AP18) (NeoMarkers, Fremont, CA) yielded the strongest MAP-2 immunoreactivity when it was used with 10mM citric acid, pH 6.0. These observations suggest that 10 min in citric acid, pH 6.0, is the optimal microwave-assisted AR method for immunolabeling MAP-2 in formalin-fixed, paraffin-processed guinea pig brain samples using MAP-2 monoclonal antibody Ab-3. This undoubtedly will have important applications in our efforts to conduct retrospective studies on archival guinea brain paraffin blocks, ultimately relaxing the use of additional animals to evaluate MAP-2 differential between chemical warfare nerve agent-treated and control samples.

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals,* National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and

Accreditation of Laboratory Animal Care International. The findings contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the U.S. Army or the Department of Defense.

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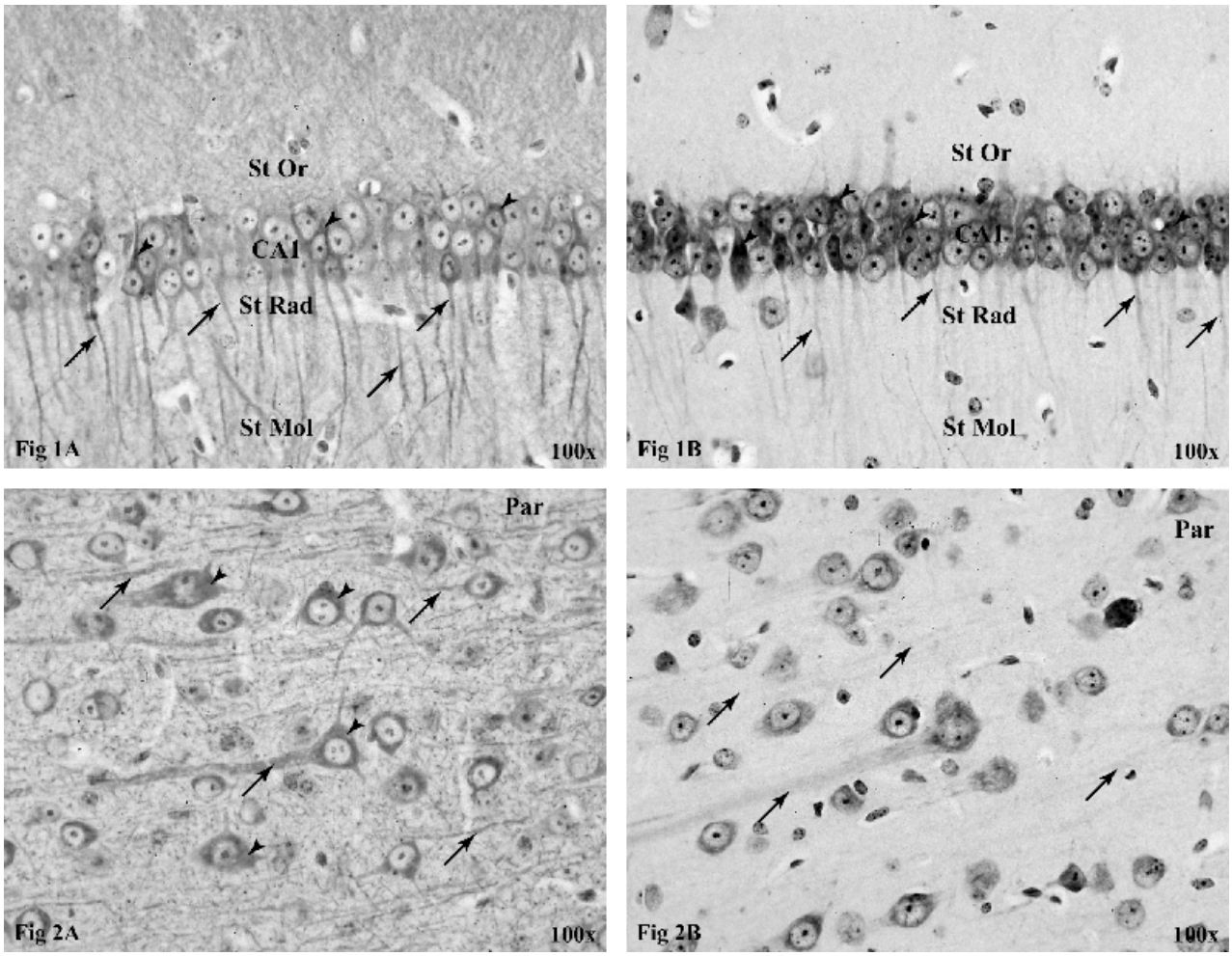

Fig. 1. Light micrograph of hippocampal sections showing MAP-2 immunoreactivity processed (A) with and (B) without microwave pretreatment. Immunoreactivity of MAP-2 is localized in neurons (arrowheads) and dendrites (arrows). St Or, Stratum Oriens; CA1, Cornus ammons 1; St Rad, Stratum Radiatum; St Mol, Stratum Moleculare.

Fig. 2. Light micrograph of cortical sections showing MAP-2 immunoreactivity processed (A) with and (B) without microwave pretreatment. Immunoreactivity of MAP-2 is localized in neurons (arrowheads) and dendrites (arrows). Par, Parietal cortex.

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