7 Microwave Fixation of Rat Hippocampal Slices

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INTRODUCTION

The authors' research focuses on the structural basis of synaptic function. Knowing the dimensions and connectivity of presynaptic and postsynaptic elements is necessary for understanding the mechanisms of synaptic transmission in the central nervous system. To this end, electrophysiological (EP) methods are used in combination with confocal microscopy, serial electron microscopy (EM), three-dimensional reconstruction, and quantitative image analysis. Microwave (MW)-enhanced methods for preparing hippocampal (HC) slices are described in this chapter. These procedures have been adapted from Jensen and Harris (1989) and Login and Dvorak (1985).

MW-enhanced fixation has greatly improved the integrity of slice preparation for morphological study. Earlier procedures, using immersion fixation, required 1–2 h for the aldehydes to reach the center of the slice. Thus, the interior tissue became hypoxic during fixation, which led to severe deterioration of structure. Hence, it was desirable to have a procedure that produces rapid fixation throughout the slice, without additional manipulation of the slice prior to immersion fixation (Chang and Greenough, 1984; Harris et al., 1980; Petukhov and Popov, 1986; Reid et al., 1988).

HC slices are prepared for EP by a variety of approaches, depending on the specific goals of the experiments (Harris and Teyler, 1984; Jackson et al., 1993; Sorra and Harris, 1998; Kirov et al., 1999). The authors

From: *Microwave Techniques and Protocols*
Edited by: R. T. Giberson and R. S. Demaree Jr.

have based these methods on living slices that have been maintained at the interface of oxygenated air (95% O_2, 5% CO_2) and artificial cerebral spinal fluid components within a chamber. The best depth for recording EP responses in brain slices is between 70 and 150 µm below the air surface (Anderson et al., 1980; Jensen and Harris, 1989). Therefore, high-quality preservation of ultrastructure at this depth within the slice has been the main objective. The MW-enhanced fixation process produces high quality fixation to the center of the slice within seconds, so that one can detect those morphological changes that are likely to be associated with an experimental manipulation, as opposed to the effects of hypoxia during slow immersion fixation (Harris and Jensen, 1989).

HC slices destined for EM undergo further processing, according to the schedule discussed below. Four d are needed for routine immersion processing, but only 3–4 h are required when MW-enhanced processing is used. Representative examples of tissue preserved from MW-enhanced fixation and perfusion-fixed HC in vivo are compared, and the processing details are presented. In addition, the authors demonstrate that resectioning the slices to 100 µm provides more uniform penetration of reduced osmium (Os) fixative, and gives the most densely stained membranes, which is desirable for tracing dendrites and synapses through serial thin sections. These methods have been used primarily for HC slices and perfusion-fixed hippocampus in vivo (Sorra and Harris, 1998; Shepherd and Harris, 1998; Fiala et al., 1998; Jensen and Harris, 1989; Kirov et al., 1999), but the authors anticipate that they will prove to be applicable to all brain and spinal cord slice preparations, with slight modifications (White et al., 1994; Feirabend et al., 1994).

MATERIALS AND METHODS

MWX-Enhanced Fixation of Slices Maintained In Vitro

1. Slices are prepared according to standard procedures for EP or pharmacological studies (Harris and Teyler, 1984; Jackson et al., 1993; Sorra and Harris, 1998; Kirov et al., 1999).
2. The HCs are removed from rats of various developmental ages, ranging from birth to young adult (65–75 d old).
3. 4–6 transverse slices (400 µm) are cut from the middle-third, with a tissue chopper (Stoelting, Wood Dale, IL) or vibroslicer, VT1000S (Leica, Microsystems Inc., Bannockburn, IL), into ice-cold media containing (in m*M*) ~117 $NaCl_2$, ~5.4 KCl, ~26.2 $NaHCO_3$, 1 NaH_2PO_4, either 2.5 or 3.2 $CaCl_2$, ~1. 6 $MgSO_4$, and 10 glucose, equilibrated with 95% O_2–5% CO_2, pH 7.4.

4. Slices are transferred to nets positioned over wells, with physiological saline at the interface of humidified 95% O_2–5% CO_2, at 32°C, in a recording chamber (Stoelting), and maintained for varying times in vitro.
5. EP recordings are made according to experimental protocols (Harris and Teyler, 1984; Jackson et al., 1993; Kirov et al., 1999; Sorra and Harris, 1998).
6. At the end of each experiment, the electrodes are removed.
7. The slice, still attached to its net, is quickly transferred (less than 30 s) to 5.5 mL fixative (6% glutaldehyde, 2% paraformaldehyde, 2 m*M* $CaCl_2$, 4 m*M* $MgCl_2$ in 0.1 *M* cacodylic buffer at ~23°C) in a 35-mm polystyrene Petri dish.
8. The dish is placed in a vented MW oven (Amana Radar Range, model RS4141, 700 W), situated immediately near the recording rig.
9. Prior to experimentation, empirical procedures were used to calibrate the duration for MW-enhanced fixation. Slices are exposed to microwave irradiation (MWI), with fixative for increasing duration until an optimal temperature (temp) (35–58°C) is achieved. The temp is measured immediately after MW-enhanced fixation is complete. The final temp should not exceed 58°C, otherwise microtubules are lost. In the Amana MW oven, the optimal time is 8 s at full power in a MW cold spot, as determined with the neon bulb array (Giberson and Demaree, 1995; Jensen and Harris, 1989). Two water loads (800 mL each, in two beakers) are placed in the rear corners of the oven, to absorb reflected radiation.
10. Postfixation, up to an overnight period, after MW fixation in the same fixative, is not needed (Jensen and Harris, 1989); however, it is convenient, and serves to stabilize the tissue for subsequent handling.
11. Slices are transferred to 0.1 *M* cacodylic buffer for several rinses, and are stored in this buffer prior to further tissue processing.

This procedure is optimized for 400-μ thick brain slices. However, if larger tissue samples are required, such as for uncut HC or another portion of the brain, the authors have found that steps 8 and 9 can be modified slightly. Namely, the 35-mm dish with 5.5 mL fixative is placed on a large bag of crushed ice, and microwaved for 1 min. The water loads are still present in the back of the MW oven. This procedure allows a longer exposure to the MW energy, to facilitate penetration of the aldehydes through the thicker tissue while maintaining a temp low enough so that the tissue at the surface is not overheated and destroyed.

MW-Enhanced Processing

MW-enhanced processing is done in a programmable Pelco model 3450 (Ted Pella) MW oven with the Pelco 3420 load cooler attachment and temp probe. This MW oven is kept in a fume hood.

1. Embed the fixed 400-μm slice (or larger piece of tissue) in agar, and trim manually, under a dissecting scope, to isolate the region of interest (e.g., HC area CA1) for processing.
2. Re-embed the region of interest in agar, and section at 100 μm, using the Leica VT1000S vibrating slicer, keeping the central two pieces of a 400-μm slice. This procedure removes the outer 150–200 μm of cut surface, where the tissue is damaged.
3. Rinse, and store sections in 0.1 *M* cacodylic buffer.
4. Place sections and buffer in 1.7 mL polypropylene microcentrifuge tubes (MTs) positioned in a rack around a central water-filled MT, which serves as a temp monitor during MWI.
5. Position MTs in a designated cold spot. The rationale and calibration for optimal sample placement and strategic positioning of the two water loads within the MW oven has been previously described (Giberson and Demaree, 1995; Giberson et al., 1997).
6. Each water load contains 400 mL distilled water in a polypropylene beaker. One water load is always connected to the load cooler attachment (Ted Pella) via inlet and outlet tubes in the oven cavity. The water in the other beaker is changed frequently throughout the MW sessions, to avoid overheating and loss of MW-absorption capacity.
7. The volume amounts of all processing chemicals in steps 8–12 below are 600 μL in the MTs (Giberson et al., 1997). The tip of the temp probe is positioned 2 mm deep in the 600 μL water in the monitor tube.
8. Remove buffer from tissue and add 1% Os and 1.5% potassium ferrocyanide in 0.1 *M* cacodylic buffer, and cool on an ice bath, until the temp in the monitor tube is less than 15°C. Then MWI for 2.5 min, up to 37°C.
9. Rinse tissue in 5–6 changes of 0.1 *M* cacodylic buffer, outside of MW oven, 1 min/change.
10. Add 1% osmium in 0.1 *M* cacodylate buffer, cooled to less than 15°C on ice, and MWI for 2.5 min, up to 37°C.
11. Rinse tissue in 5–6 changes of 0.1M cacodylic buffer, outside of MW oven, 1 min/change. Rinse tissues again, but in two brief changes of distilled water.
12. Stain *en bloc* with 1% aqueous uranyl acetate cooled on ice to less than 15°C, and MWI for 2.5 min, up to 37°C, followed by two brief water rinses.
13. For steps 14–17 below, add 15–20 mL fresh acetones or resins in the 60-mm polypropylene petri dishes and position the temp probe 4 mm deep in acetone or resin in the 60-mm Petri dish (Giberson et al., 1997). Failure to maintain the probe 4 mm deep in resin results in premature polymerization, (R. Giberson, personal communication).
14. Transfer slices to the flowthrough baskets seated in 60-mm Petri dishes containing 50% acetone (aqueous).

15. Dehydrate through graded acetone series (50, 70, 90 and 100%, two changes each), and MWI for 40 s during each change, up to 37°C.
16. Infiltrate slices in the flowthrough baskets with 1 acetone: 1 Spurr's-Epon (1 Spurr's: 1 Epon) resin (Ted Pella and Ladd Research, Burlington, VT), with the temp restriction level raised to 45°C and the temp probe tip submersed 4 mm deep in the resin. MWI for 15 min.
17. Infiltrate with two changes of 100% Epon–Spurr's and MWI for 15 min each change.
18. Transfer into fresh 100% resin (Epon–Spurr's or Epon alone) in either flat, coffin-shaped molds or BEEM™ capsules, then cure for 48 h in a 60°C oven.
19. Alternatively, embed in Epon–Spurr's in Pelco capsules (Ted Pella), and cure in the Pelco 3450 MW oven for 75 min according to procedures described in Giberson et al. (1997).

Microtomy

1. The cured blocks are cut with a diamond Histo knife (Diatome, US) on a Reichert Ultracut S (Leica) ultramicrotome, to obtain a full-face section of 1-μm thickness.
2. The 1-μm sections are dried onto glass slides, and stained with 1% toluidine blue stain at ~60°C, until the edges of the stain become irridescent, then are rinsed with distilled water, followed by 70% ethyl alcohol, then water-rinsed again, dried, and examined under a light microscope. The 1% toluidine blue stain is made from equal amounts of 1% toluidine blue (aq) to 1% sodium borate (aq), diluted in half with 70% ethanol.
3. A raised rectangular or trapezoidal area, usually less than 100 × 100 μm, is made with a diamond square or wedge-shaped trim tool (Diatome), in the desired area of the block face.
4. Serial thin sections are cut with a diamond knife (Diatome).
5. The serial sections are mounted on Pioloform-coated (SPI Supplies, Westchester, PA) slot grids (Synaptek, Ted Pella). To prepare the grids, ~1.2% Pioloform in chloroform is coated onto glass microscope slides, either by hand or under controlled-release, using the EFFA film caster (Ernest Fullam, Latham, NY). These coated slides are dried over desiccant for 1 min, then the Pioloform film is released from the edges with a razor blade, and floated onto water. Slot grids are gently pressed onto the surface of the Pioloform, picked up on a clean Parafilm® coated glass slide, and stored in a dry place under glass.
6. Grids containing serial sections are stained in a small glass (~60 mm diameter)-covered Petri dish containing ~3 mm dental wax, melted and cooled in the bottom of the dish. The dental wax has several rows of slits,

prepared with a clean razor blade. Each grid is partially inserted on one edge into a slit, without damaging the Pioloform coating. Either filtered, saturated uranyl acetate (aq) alone or mixed 1:1 with 100% ethanol, is added to the grids for 5 min. The stain is then poured off, and grids are rinsed with a gentle stream of twice-distilled water, for several minutes.

7. The grids are wicked dry with filter paper and then Reynold's lead stain is applied for 5 min. To reduce excess CO_2 contamination, a 5.5-cm-diameter filter paper, moistened with 0.02 *N* NaOH, is adhered to the inner side of the glass Petri dish cover. The stain is then poured off, and grids are gently rinsed with 0.02 *N* NaOH, followed by twice distilled water and wicked dry. Grids are allowed to air-dry thoroughly.
8. Each grid is loaded into a gimbol set (Advanced Microscopy Techniques, Danvers, MA), or glued to a hexagonal ring (Gatan Inc., Warrendale, PA) and stored in a labeled gelatin capsule.

Serial Section Electron Microscopy

Serial thin sections of uniform section thickness are desired for accurate measurement of synapses in three dimensions. Many factors will affect the uniformity of section thickness (Hyatt, 1989). The diamond trimming tools can be used to reliably prepare a small trapezoid for serial thin-sectioning. The square-shaped trim tool produces a rectangular mesa, resulting in serial sections that are exactly the same size. The 45° angled trim tool produces a pyramid, but the sections become sequentially larger, thereby making it more difficult to map the location of objects through serial sections during photography. Of the two, however, the angled trim tool produces sections with more uniform section thickness. Tissue, cured in either cylindrical BEEM capsules or coffin-shaped, flat molds, is used, depending on the orientation of the tissue.

Anecdotally, the cylindrical blocks seem to be held more securely in a microtome chuck, and are somewhat more reliable in producing uniform section thickness. However, BEEM capsules are not convenient for orienting tissue perpendicularly to the broad plane, which is needed in many of the authors' studies. Another consideration is the type of resin the tissue is in (Epon vs Epon–Spurr's). These sectioning-related issues continue to be evaluated in relationship to MWI curing of the Epon. In the authors' experience, blocks cured with MWI can achieve uniform section thickness of a quality equal to those slowly cured for 48 h at 60°C (e.g., Fig. 1).

To view a series of consecutive thin sections (~60 nm section thickness) in the same orientation, grids in their gimbols are placed in a rotating-stage specimen holder (EM-SRH, Jeol USA, Peabody, MA) in

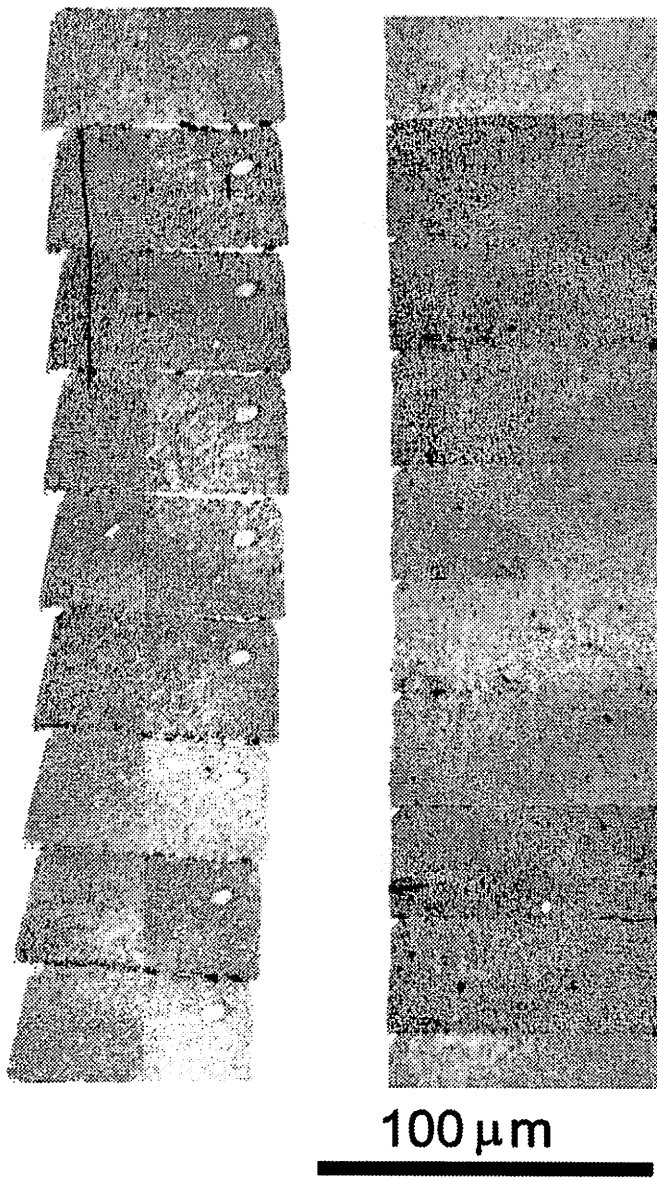

Fig. 1. Portions of two ribbons from serial sections. The ribbon on the left is from a series prepared by conventional methods, and the series on the right was prepared by MW-enhanced fixation and processing. Although there are some irregularities in each of these ribbons, they demonstrate that the MW processing is comparable to conventional methods.

the Jeol 1200EX electron microscope (JEOL USA, Inc.). In the same manner, a grid, attached to a Gatan hexagonal ring is placed in the Gatan model 650 rotational holder (Gatan, Inc.) and can be viewed in the JEOL 2010 electron microscope. The grid is rotated as needed, to maintain a reference fiduciary (such as a large cross-sectioned dendrite) in the center of the field. Alternatively, visual maps are drawn and edited as needed, to maintain several objects in the same positions relative to one another across serial sections. A calibration grid (Ernest Fullam) is photographed with each series. Section thickness *(st)* is determined by measuring the diameters *(d)* of longitudinally sectioned tubular objects (such as dendrites, mitochondria, or axons) in the micrographs (x-y plane), and counting the number of sections (n) that they span (z-axis), and calculating $st = d/n$, which is averaged across many measurements obtained throughout the series (e.g., *see* Shepherd and Harris, 1998).

RESULTS

Only about 50 μm surrounding the periphery of a tissue block is consistently penetrated by ferrocyanide–reduced Os (Hayat, 1989; Fig. 2A). Potassium ferricyanide—reduced osmium also exhibited a similar limit in stain penetration. The authors found, however, that, if the HC slice was resectioned to 100 μm or less, the neuropil was uniformly stained throughout the section (Fig. 2B).

In slice preparations, the quality of the tissue improves in a graded manner, from the cut surfaces toward the middle slice (Fig. 2A; *see also* Jensen and Harris, 1989). Near the cut surface (Fig. 3A), there are dark, degenerating processes and vacuolated structures indicative of trauma caused by the cutting of the slices (Loberg and Torvik, 1993). Zones of excellent ultrastructure are found at ~100–200 μm beneath the slice surface, in conjunction with the best EP (Fig. 2B). Excellent ultrastructural preservation of the neuropil is recognized by intact cytoplasmic and nuclear membranes, uniformly thick postsynaptic densities, a uniform distribution of presynaptic axonal vesicles, distinct microtubules, absence of vacuolation or shrinkage artifacts, and intact mitochondria. Similar results are published elsewhere (Harris et al., 1992; Kirov et al., 1999; Shepherd and Harris, 1998; Sorra and Harris, 1998).

If a 400-μm-thick piece of tissue is processed, then, as one moves toward the center of the tissue, the density of staining falls off precipitously (Fig. 2A), so that the region of interest in the center of the block is not well-stained (Fig. 3C,D). This problem was completely overcome

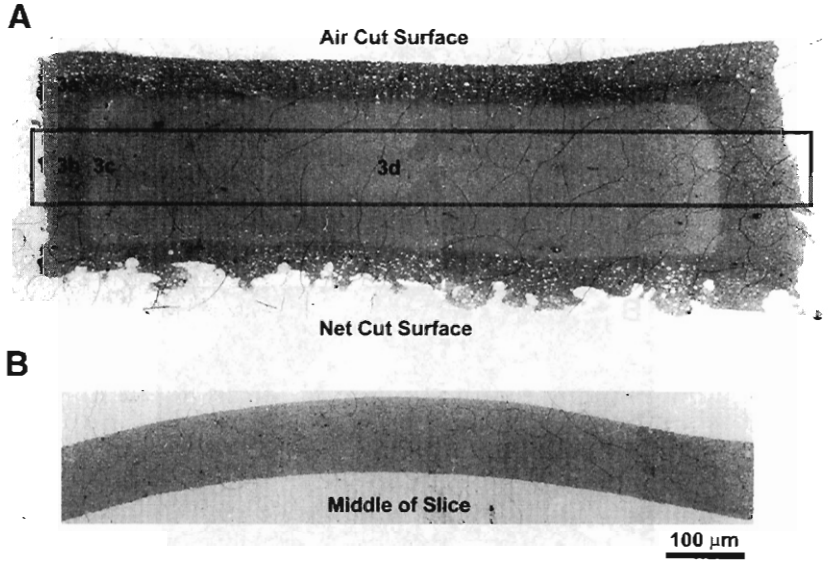

Fig. 2. Improvement in staining quality by reducing the thickness of the tissue. **(A)** 400-µm-thick HC slice. The "net cut surface" of the slice rested down on a net; the "air cut surface" was at the interface of the media and oxygenated, humidified air. The gray rectangle illustrates the region of optimal tissue quality that is sufficiently far from the cut edges, so that there is little or no damage to the processes (*see* Figs. 3 and 4). **(B)** 100-µm section from the middle of another 400-µm HC slice, which was resectioned to achieve complete penetration of the reduced Os to the region of interest. Both sections were processed by MW-enhanced methods, and photographed at ×60 in the Jeol EM. They are not toluidine blue stained; hence, the rim around the outer edge of the thicker slice in (A) results from the incomplete penetration by reduced Os.

by resectioning the slice to 70–100 µm thickness: Then, all portions of the region of interest have uniformly high-intensity staining of the plasma membranes (Fig. 4A–C). The quality of the neuropil in these sections is comparable to or better than, that obtained from perfusion-fixed brain processed by standard methods.

DISCUSSION

MWI during tissue fixation and processing has greatly facilitated research (Kirov and Harris, 1999; Fiala et al., 1998; Shepherd and Harris, 1998; Sorra and Harris, 1998). The rapid fixation produces superior tissue preservation in HC slices; MW-enhanced processing has greatly

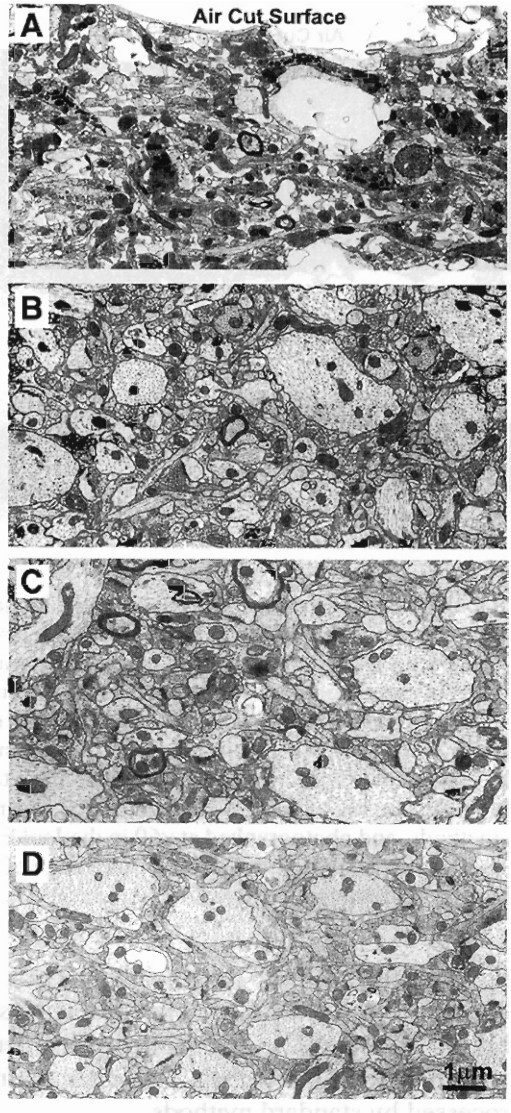

Fig. 3. Photographs from the 400-μm slice in Fig. 2A. (**A**) Damaged tissue near to the air cut surface of the HC slice. More cells die at the net surface, because it is less well oxygenated. All photographs in (**B–D**) are located 144 μm from the air-cut surface of the slice, in the region of optimal tissue quality, as demonstrated both physiologically and morphologically. However, photographs in C and D have lower membrane staining, because of the lack of penetration by the reduced Os. The lower contrast and staining quality can result in broken membranes and less-optimal membrane staining for serial EM.

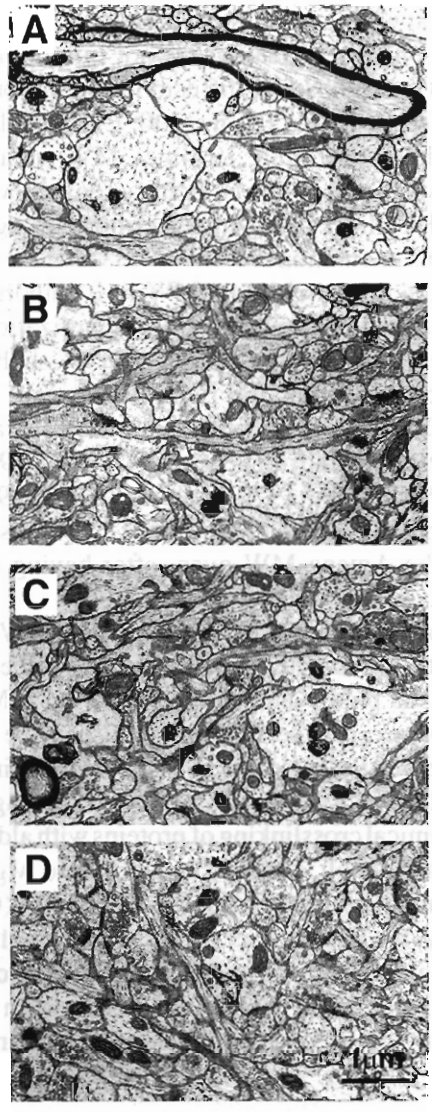

Fig. 4. All images photographed from the center of a resectioned HC slice are of high contrast and optimal staining quality of biological membranes through serial EM sections. Pictures in **(A–C)** are from the center of a 100-µm section through the middle of a HC slice, from a postnatal d 21 rat, fixed and processed by the enhanced MW procedures. The picture in **(D)** is from a perfusion-fixed postnatal d 21 rat processed by enhanced MW procedures, although using a 400-µm section. This picture is from the edge of the section near where some of the reduced Os would have penetrated the tissue section, and is similar to Fig. 2C.

speeded the rate at which the authors achieve results. The mechanisms underlying MW-enhanced fixation are not fully understood, but they apparently result from enhanced diffusion and reaction rates, facilitated by internal heating (Kok and Boon, 1990; Evers et al., 1998; Horobin, 1998; Login and Dvorak, 1985; 1994b,c). MW-enhanced fixation has set new, higher standards in this laboratory for achieving quality tissue preservation in HC slices following electrophysiological and/or pharmacological experimentation.

When using the household-type MW, it is important to follow the guidelines for careful calibration and standardization for fixation of biological tissues. Many factors can affect MWI, such as the unpredictability of MW power output and field distribution, nonuniformity of MW heating within the oven cavity, the position of water loads, sample size, fixative volume, and so on (Login and Dvorak 1994a, 1994b, 1994c; Login et al., 1998; Login, 1978; Kok and Boon, 1992). The authors overcame these potential problems by using the exact same spot (marked by an "X") in the Amana MW oven, after having calibrated it, and testing that it gave good tissue quality.

Postfixation of the slice in aldehyde, following MWI, is another issue. Although the authors found that no postfixation of the slice is necessary for excellent tissue preservation following 8–11 s MWI (Jensen and Harris, 1989), preserving the slice for 1–12 h in the mixed aldehydes serves to stabilize the tissue during subsequent handling. Several investigators contend that postfixation of tissue, following MWI, is critical for complete chemical crosslinking of proteins with aldehydes (Mizuhira et al., 1990; Ohtani et al., 1990). The $CaCl_2$ and $MgCl_2$ also serve to maintain membrane integrity during MWI fixation (Ohtani, 1991).

Until recently, the tissue-processing for EM involved a long procedure lasting several days. The programmable MW oven designed for laboratory use (Pelco 3450, 900 W, 2.45 GHz, with model 3420 load cooler attachment), has reduced the time required for this procedure to a few hours.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants, NS21184, NS33574, MH/DA57351 which is funded jointly by National Institute of Mental Health, National Institute of Drug Abuse, National Aeronautics and Space Administration (K.M.H.), and the Mental Retar-

dation Research Center grant P30-HD18655 (Dr. Joseph Volpe, PI). The authors thank Alex Goddard for preparing some of the HC slices and Betsy Velazquez for assistance in preparing the manuscript, and Dr. John Fiala for editorial assistance.

REFERENCES

Andersen P, Silfvenius H, Sundberg SH, Sveen O (1980) Comparison of distal and proximal dendritic synapses on CA1 pyramids in guinea-pig hippocampal slices in vitro. *J Physiol* (Lond.) **307**:273–299.

Chang FLF, Greenough WT (1984) Transient and enduring morphological correlates of synaptic activity and efficacy change in the rat hippocampal slice. *Brain Res* **309**:35–46.

Evers P, Uylings HB, Suurmeijer AJ (1998) Antigen retrieval in formaldehyde-fixed human brain tissue. *Methods* **15**:133–140.

Feirabend HK, Kok P, Choufoer H, Ploeger S (1994) Preservation of myelinated fibers for electron microscopy: a qualitative comparison of aldehyde fixation, microwave stabilisation and other procedures all completed by osmication. *J Neurosci Methods* **55**:137–153.

Fiala JC, Feinberg M, Popov V, Harris KM (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *J Neurosci* **18**:8900–8911.

Giberson RT, Demaree RS, Jr. (1995) Microwave fixation: understanding the variables to achieve rapid reproducible results. *Microsc Res Tech* **32**:246–254.

Giberson RT, Demaree RS Jr., Nordhausen RW (1997) Four-hour processing of clinical/diagnostic specimens for electron microscopy using microwave technique. *J Vet Diagn Invest* **9**:61–67.

Harris KM, Cruce WLR, Greenough WT, Teyler TJ (1980) A Golgi impregnation technique for thin brain slices maintained in vitro. *J Neurosci Methods* **2**:363–371.

Harris KM, Teyler TJ (1984) Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *J Physiol* (London) **346**:27–48.

Harris KM, Jensen FE, Tsao B (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *J Neurosci* **12**:2685–2705.

Hayat MA (1989) Positive staining. In: *Principles and Techniques of Electron Microscopy*. CRC Press, Boca Raton, FL, pp. 272, 273.

Horobin RW (1998) Problems and artifacts of microwave accelerated procedures in neurohistotechnology and resolutions. *Methods* **15**:101–106.

Jackson PS, Suppes T, Harris KM (1993) Stereotypical changes in the pattern and duration of long-term potentiation at postnatal days 11 and 15 in the rat hippocampus. *J Neurophysiol* **70**:1412–1419.

Jensen FE, Harris KM (1989) Preservation of neuronal ultrastructure in hippocampal slices using rapid microwave-enhanced fixation. *J Neurosci Methods* **29**:217–230.

Kirov SA, Sorra KE, Harris KM (1999) Slices have more synapses than perfusion-fixed hippocampus from both young and mature rats. *J Neurosci* **19**:2876–2886.

Kirov SA, Harris KM (1999) Dendrites are more spiny on mature hippocampal neurons when synapses are inactivated. *Nat Neurosci* **2(10)**:878–883.

Kok LP, Boon ME (1992) *Microwave Cookbook for Microscopists.* Coulomb, Leiden, The Netherlands.

Kok LP, Boon ME (1990) Microwaves for microscopy. *J Microsc* **158:**291–322.

Loberg EM, Torvik A (1993) Distinction between artefactually shrunken and truly degenerated 'dark' neurons by in situ fixation with microwave irradiation. *Neuropathol Appl Neurobiol* **19:**359–363.

Login GR (1978) Microwave fixation versus formalin fixation of surgical and autopsy tissue. *Am J Med Technol* **44:**435–437.

Login GR, Dvorak AM (1985) Microwave energy fixation for electron microscopy. *Am J Pathol* **120:**230–243.

Login GR, Dvorak AM (1994a) Application of microwave fixation techniques in pathology to neuroscience studies: a review. *J Neurosci Methods* **55:**173–182.

Login GR, Dvorak AM (1994b) Methods of microwave fixation for microscopy. A review of research and clinical applications: 1970–1992. *Prog Histochem Cytochem* **27:**1–127.

Login GR, Dvorak AM (1994c) The Microwave Tool Book. Beth Israel Hospital, Boston.

Login GR, Leonard JB, Dvorak AM (1998) Calibration and standardization of microwave ovens for fixation of brain and peripheral nerve tissue. *Methods* **15:**107–117.

Mizuhira V, Notoya M, Hasegawa H (1990) New tissue fixation methods for cytochemistry using microwave irradiation. I. General remarks. *Acta Histochem Cytochem* **23:**501–523.

Ohtani H, Naganuma H, Nagura H (1990) Microwave-stimulated fixation for histocytochemistry: application to surgical pathology and preembedding immunoelectron microscopy. *Acta Histochem Cytochem* **23:**585–597.

Ohtani H (1991) Microwave-stimulated fixation for preembedding immunoelectron microscopy. *Eur J Morphol* **29:**64–67.

Petukhov VV, Popov VI (1986) Quantitative analysis of ultrastructural changes in synapses of the rat hippocampal field CA3 in vitro in different functional states. *Neuroscience* **18:**823–835.

Reid KH, Edmonds HL, Jr., Schurr A, Tseng MT, West CA (1988) Pitfalls in the use of brain slices. *Progr Neurobiol* **31:**1–18.

Shepherd GMG, Harris KM (1998) Three-dimensional structure and composition of CA3—>CA1 axons in rat hippocampal slices: implications for presynaptic connectivity and compartmentalization. *J Neurosci* **18:**8300–8310.

Sorra KE, Harris KM (1998) Stability in synapse number and size at 2 hr after long-term potentiation in hippocampal area CA1. *J Neurosci* **18:**658–671.

White EL, Amitai Y, Gutnick MJ (1994) Comparison of synapses onto the somata of intrinsically bursting and regular spiking neurons in layer V of rat SmI cortex. *J Comp Neurol* **342:**1–14.