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The histochemical localization of cytochrome oxidase in the dentate gyrus of the rat hippocampus

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In the dentate gyrus of the rat's hippocampal formation, the activity of an oxidative enzyme, cytochrome oxidase, has been localized mostly to the molecular layer with histochemical methods that utilize diaminobenzidine. The electron microscopic localization of cytochrome oxidase indicated that mitochondria within granule cell dendrites were very reactive while those within the somata and mossy fiber terminals of this neuronal type were less reactive. Caution must be used when predicting the relative physiological activities of neurons with this method because differential activities of this enzyme occur within separate parts of the same neuronal population.

Cytochrome oxidase activity in areas of the brain can be demonstrated by incubating sections in buffered media consisting of 3,3' diaminobenzidine (DAB) and either cytochrome C or hydrogen peroxide^{7–10}. The DAB is oxidized by cytochrome C or hydrogen peroxide to form a polymeric reaction product at sites of cytochrome oxidase activity that have been shown to be located on the outer surfaces of the inner mitochondrial membranes⁵. Using this method, Wong-Riley and colleagues^{8–10} found that an experimentally induced decrease in neuronal activity led to a significant decrease in the level of cytochrome oxidase activity. Thus, it was suggested that a correlation existed between cytochrome oxidase activity and neuronal activity.

In the present study, cytochrome oxidase activity was investigated in the dentate gyrus (DG) of the rat hippocampus in order to determine the relative neuronal activities of two types of DG cells, pyramidal basket (PB) cells and granule cells. The cell bodies of these neurons lie in two adjacent layers of the DG. The smooth dendrites of the PB cells project through the granule cell layer to arborize in the molecular layer with the spine-bearing dendrites of the granule cells¹. The PB cell bodies have unique ultrastructural characteristics that are different from the granule cells³. Some of these features, such as highly infolded, euchromatin-containing nuclei and intranuclear rods, suggest that the PB cells have higher neuronal activities than the granule cells³. Since these DG cells have not been analyzed in intracellular physiological studies, a histochemical localization of cytochrome oxidase may be able to demonstrate these suggested differences in neuronal activities. Therefore, the present study was undertaken to show if any differences in cytochrome oxidase activity exist between the PB and granule cells.

Seven, anesthetized Sprague–Dawley rats were perfused intracardially with an initial saline washout followed by a solution of 4% paraformaldehyde and 1% glutaraldehyde in a 0.12 M phosphate buffer. Before the perfusions began, the rats were artificially respired with a tracheal tube, and received intracardiac injections of sodium nitrite (0.9 ml) and heparin (0.1 ml) to inhibit clotting and vasodilate blood vessels. Brains remained overnight in situ and were dissected the following day. Coronal sections of the DG were cut on a Sorvall TC-2 tissue sectioner at 70 μm . Tissue sections were rinsed 3 times for 10 min each in a 0.12 M phosphate buffer before incubating in either of two media⁹. The first medium consisted of 0.2% 3-3' diaminobenzidine and 0.02% hydrogen peroxide in a 0.05 M 2-amino-2-methyl-1,3-propanediol buffer at pH 9.0. The second medium consisted of 0.06% 3-3' diaminobenzidine, 0.06% cytochrome C type III (Sigma) and 4.5% sucrose in a 0.12 M phosphate buffer at pH 7.4.

Tissue sections incubated in either medium were kept at 37 °C for 1–2 h until a reaction product was formed. Sections from the cerebellum and cochlear nucleus were also incubated. In addition, a control incubation medium was used for DG sections and it contained 0.01 M potassium cyanide, a powerful inhibitor of metalloenzymes, especially the iron-containing cytochromes. Following the incubation, tissue sections were rinsed 3 times in 0.1 M phosphate buffer, dehydrated, and mounted for light microscopic observation. In addition, other incubated tissue sections from the dentate

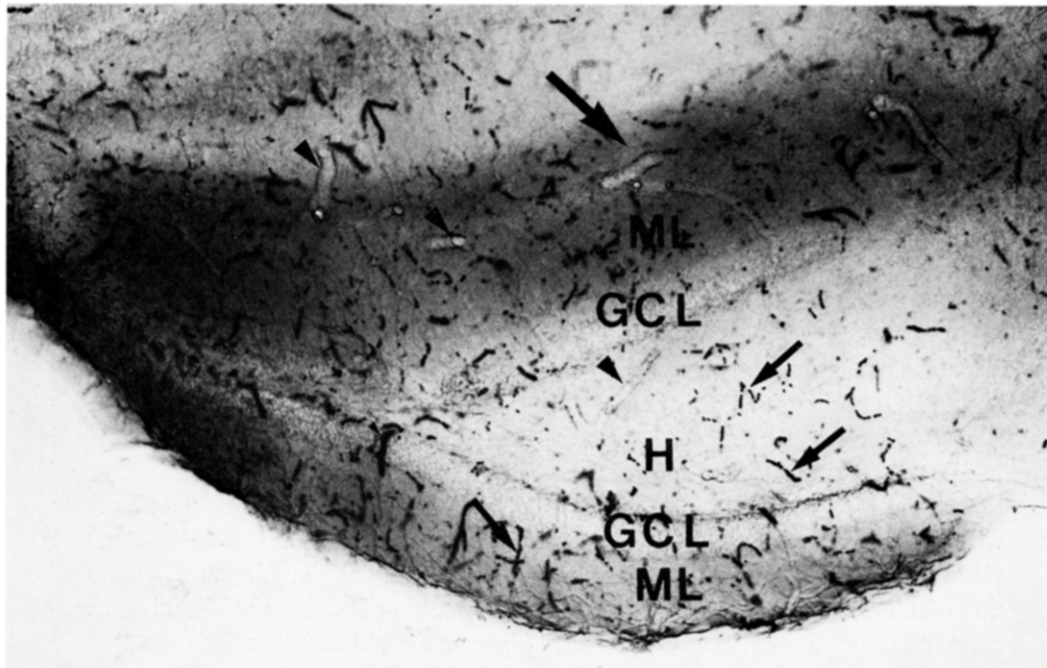


Fig. 1. Low power light micrograph of dentate gyrus showing cytochrome oxidase activity. The greatest staining occurs in a band found within the molecular layer (ML) while less staining occurs in the granule cell layer (GCL) and hilar region (H). The molecular layer of the hippocampus (large arrow) also shows an increased amount of cytochrome oxidase activity. The red blood cells within small capillaries (small arrows) are also stained but the larger blood vessels are clear (arrowheads). $\times 100$.

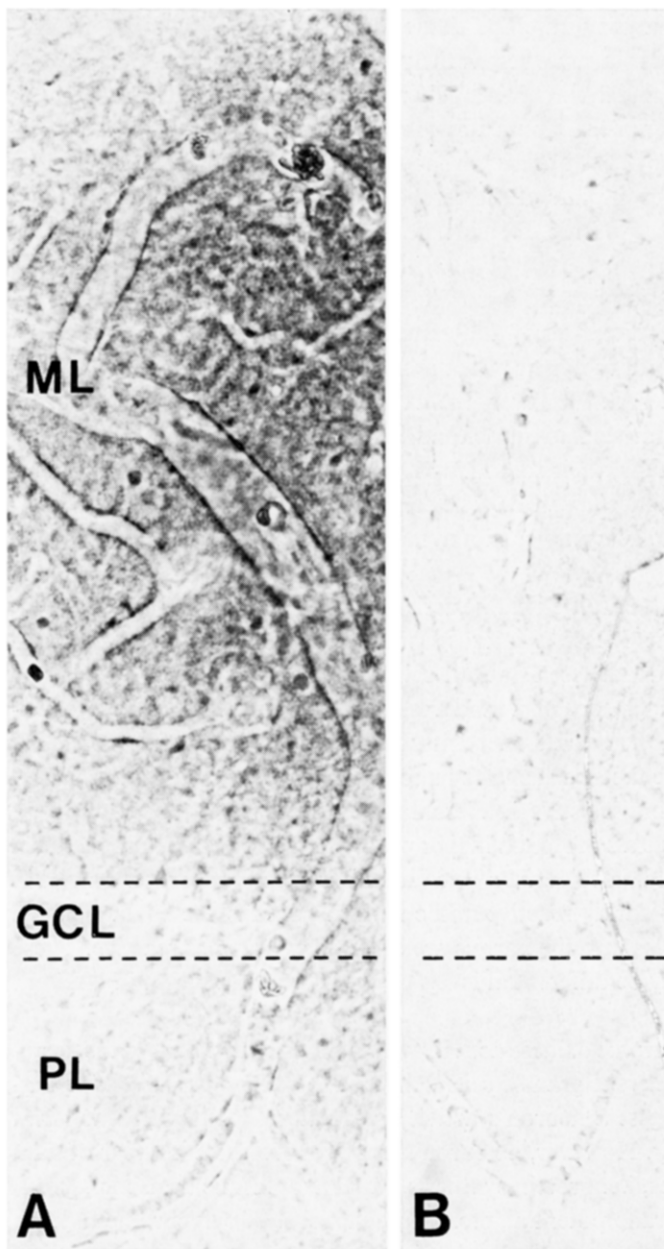


Fig. 2. Light micrographs of sections of dentate gyrus showing specific staining pattern of cytochrome oxidase activity. A: band of cytochrome oxidase activity in the molecular layer (ML). The granule cell (GCL) and polymorph (PL) layers are relatively unstained. B: section incubated in the potassium cyanide control that lacks cytochrome oxidase activity. Both A and B are taken with phase contrast optics. $\times 400$.

gyrus, cerebellum, and cochlear nuclei were embedded for electron microscopy using established procedures³.

Light microscopic analysis of the DG tissue showed a band of cytochrome oxidase activity in the molecular layer, whereas the polymorph and granule layers were not highly reactive (Figs. 1 and 2A). Other highly reactive areas near the dentate gyrus included the molecular layer of the hippocampus and some of the layers of the overlying cerebral cortex. Sections of the brain stem showed high cytochrome oxidase activity in the cochlear nuclei and in parts of the cerebellum, consistent with the results of Wong-Riley⁷. Control tissue of the DG incubated in 0.01 M potassium cyanide showed no cytochrome oxidase activity (Fig. 2B).

In electron microscopic preparations of the DG molecular layer, many mitochondria within the spinous dendrites of the granule cells had reaction product localized along the outer surface of the inner mitochondrial membranes, sometimes filling the intracristae space (Fig. 3A). However, the axon terminals that formed synapses with the spines of these dendrites had mitochondria that were much less reactive. The other parts of the granule cells, their somata and mossy fiber terminals, had very few mitochondria that were reactive (Fig. 3B). The somata, basal dendrites, and axon terminals of the PB cells also displayed occasional mitochondria with this dense staining (Fig. 3C).

The localization of cytochrome oxidase activity within the DG was concentrated within the molecular layer. Electron microscopic analysis demonstrated that this activity was greatest in the dendrites of granule cells, and this finding suggests that the other parts of these neurons (i.e. cell body and axon terminals) have less oxidative activity. The relatively greater activity within dendrites suggest that they may function in the active secretion of a trophic factor which may underlie the ordered plastic changes in the molecular layer following selective deafferentation². It is interesting to note that Wong-Riley and Welt¹⁰ have also found a higher proportion of reactive mitochondria within dendrites than somata. In this study¹⁰, dendrites of neurons in cortical barrels in the mouse contained many darkly-reactive mitochondria whereas the somata of the neurons which send dendrites into the barrels are themselves not very reactive. These data indicate that there may be differential levels of oxidative metabolism in different parts of a neuron.

The differential cytochrome oxidase activity within the granule cells and the decreased staining of PB cells relative to granule cells is difficult to interpret in light of Wong-Riley's results⁸⁻¹⁰ that suggest a neuron's activity correlates with its mitochondrial cytochrome oxidase activity. However, a neuron's activity involves a number of vital functions besides firing rates, i.e. protein synthesis, rapid axoplasmic transport, maintenance of a resting membrane potential, etc. Thus, the level of cytochrome oxidase activity at any given moment reflects the level of neuronal activity as defined by all of these cellular processes. Although a change in synaptic activity can cause changes in cytochrome oxidase activity⁸⁻¹⁰, a single cellular function, such as rate of firing, can not be used as the only criterion for predicting the level of cytochrome oxidase activity in different neurons under normal conditions. Therefore, the relative firing rates of PB and granule cells in the DG cannot be predicted from the cytochrome oxidase histochemical data.

The intense cytochrome oxidase activity within the molecular layer of the DG

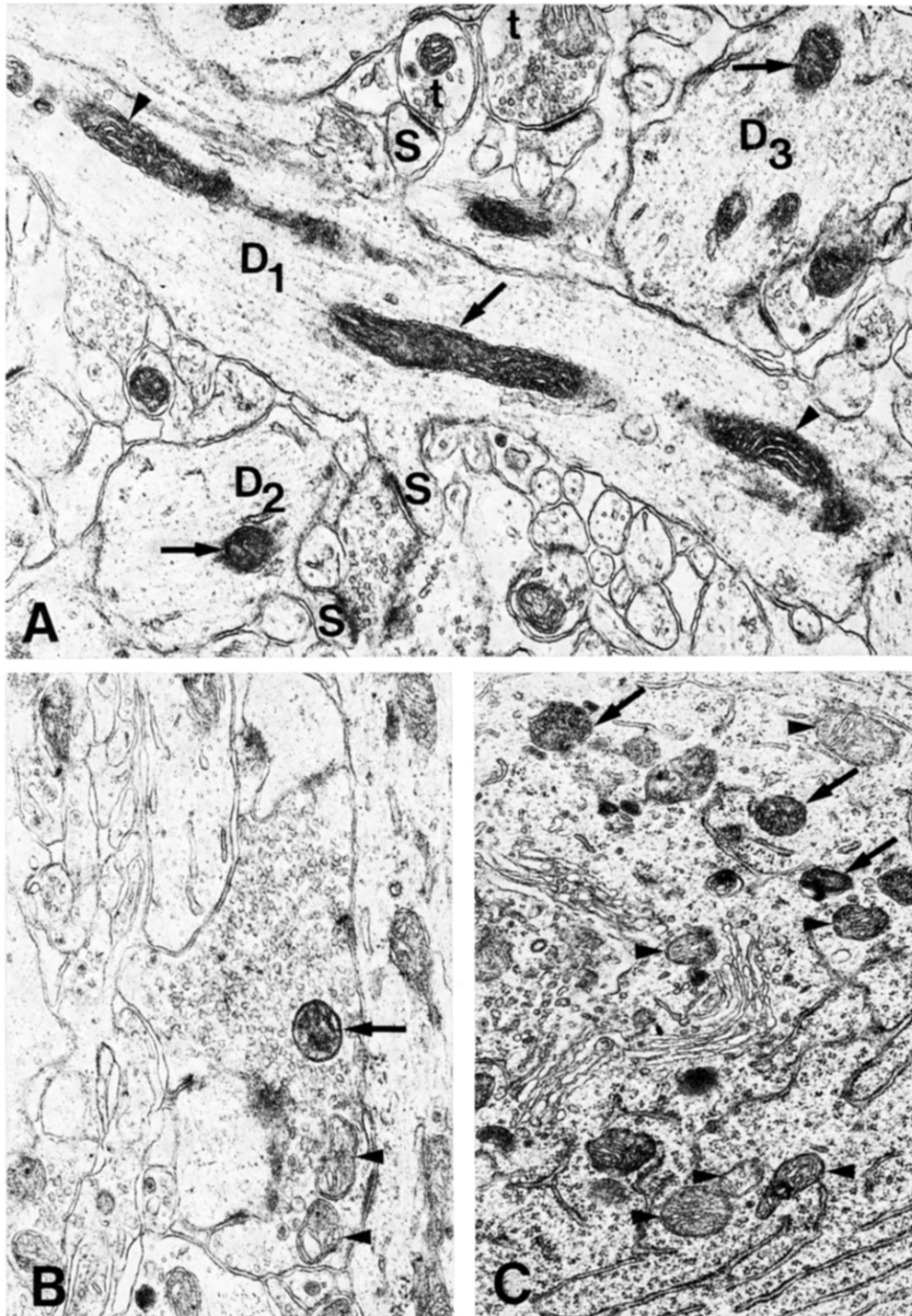


Fig. 3. Electron micrographs of sections of the dentate gyrus that have been incubated in DAB and cytochrome C. A: longitudinally sectioned (D₁) and two cross-sectioned (D₂ and D₃) dendrites with spines (S) in the molecular layer contain many reactive mitochondria with stained cristae (arrows). Two mitochondria (arrowheads) in one of these dendrites (D₁) are less reactive. In general, the mitochondria in dendrites are more reactive than those in axon terminals (t). $\times 31,000$ B: mossy fiber terminal in the polymorph layer contains two unreactive mitochondria (arrowheads) and another which is very reactive (arrow) $\times 29,000$. C: part of a pyramidal basket cell soma that is characterized by numerous cisternae of granular endoplasmic reticulum and Golgi complex³ displays more unreactive mitochondria (arrowheads) than reactive ones (arrows). $\times 20,000$

indicates a restrictive zone for oxidative metabolism in this region. This result is consistent with the results of glucose uptake experiments^{4,6} which show a higher uptake of radioactive 2-deoxyglucose in the molecular layer of the DG than in the granule cell layer. These observations further suggest that the dendritic zone may very well have a different level of oxidative metabolism than the layer of cell bodies.

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