Histochemical localization of glutamic acid dehydrogenase activity in the cuneate nucleus of the cat[1]

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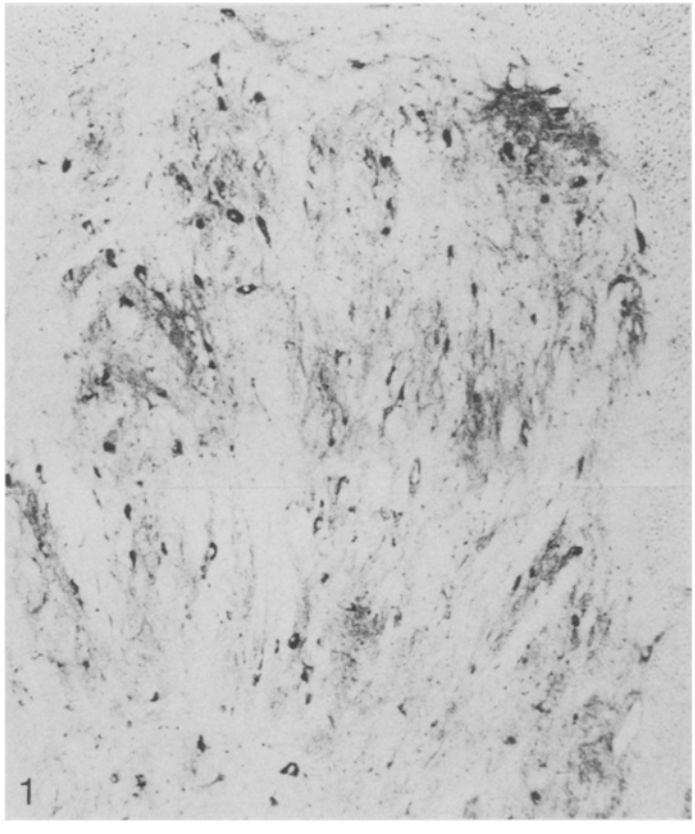
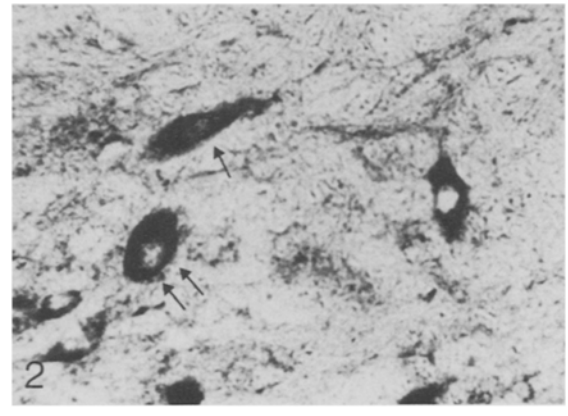
Summary. Glutamic acid dehydrogenase activity was observed both in the neuropil as well as in several classes of cuneate neurons in the cat.

Biochemical studies[2] have shown that several areas of the vertebrate central nervous system have a high content of glutamic acid. Among these areas are the dorsal root and dorsal root ganglion. Johnson and Aprison[2] have noted a high glutamate content not only in the dorsal root and dorsal root ganglion but also in the dorsal column nuclei. Several experiments have shown that when glutamic acid was iontophoretically applied to cuneate neurons, they were excited[3,4]. On the basis of these observations, glutamic acid has been implicated as a putative excitatory neurotransmitter substance in the cuneate nucleus. The role of glutamic acid in the vertebrate and invertebrate nervous system has been reviewed by Johnson[5]. On the other hand, histochemical studies of enzyme activity relating to this amino acid have been few. This paper reports the histochemical localization of glutamic acid dehydrogenase activity in the cuneate nucleus of the cat.

Material and method. Adult cats of either sex and weighing between 2.0 and 2.5 kg were used for this study. The cats were anesthetized with 0.5 ml of Sagatal (which contains 60 mg of sodium pentobarbital per ml) per kg b.wt and then rapidly perfused through the left cardiac ventricle with 1500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2–7.4. After the perfusion, the brainstem was dissected out and 30-µm transverse frozen sections were cut in a cryostat and collected in 0.1 M Tris-HCl buffer, pH 7.5. The sections were then incubated in the medium described by Martinez-Rodriguez et al.[6] for 30–45 min at 37 °C. After incubation, the sections were mounted on slides and covered with DPX and a coverslip.

Results. In the cuneate nucleus, there was moderate staining of the neuropil. The staining, however, was not evenly distributed throughout the nucleus but appeared in patches with clear intervening areas (fig. 1), probably a fixation artefact. On the other hand, cuneate neurons showing enzyme activity were very darkly stained. Only the cytoplasm was stained so that in cell profiles which contained the nucleus, the latter structure was clearly visible as an unstained area (figs 2, 3). The stained cells were distributed throughout the entire rostrocaudal extent of the nucleus.





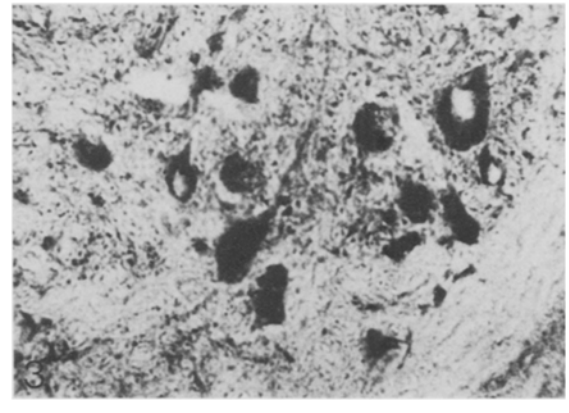


Figure 1. A low power photomicrograph of the cuneate nucleus caudal to the obex, showing patchy staining of the neuropil with intervening clear, unstained areas. Stained neurons can also be seen throughout the dorsoventral extent of the nucleus, with a greater preponderance in the dorsal half of the nucleus. × 90.

Figure 2. A high power photomicrograph showing stained fusiform cells (single arrow) and oval cell (double arrows). The nucleus is unstained. × 330.

Figure 3. A high power photomicrograph showing two small clusters of stained neurons in the dorsal half of the cuneate nucleus. The nuclei are unstained. Most of the neurons are round or oval. × 330.

However, caudal to the obex, there appeared to be more stained cells in the dorsal half of the nucleus where the 'cell nests', which were described by Keller and Hand[7], are located. In this region, many of the stained cells appeared to cluster into small groups (fig. 3) whereas elsewhere in the nucleus, they tended to lie singly.

The stained cells varied in shape. Most were round or oval and a few were fusiform (figs 2, 3). The round profiles were of 2 types: one type was medium-sized (15–18 µm in diameter) and the other type was smaller (8–12 µm). The oval profiles were larger and measured 22–26 µm in their long diameter. The fusiform cells measured 25–28 µm in their long diameter.

Discussion. The present study has demonstrated histochemically that in the cuneate nucleus of the cat, glutamic acid dehydrogenase, an enzyme which is involved in the degradation of glutamic acid to oxoglutaric acid, is located both in the neuropil and in several types of neuronal perikarya. In the neuropil, staining was slightly more intense in the dorsal part of the nucleus where primary afferent fibers of dorsal root origin have been shown to terminate predominantly[7], than in the ventral part. This would correlate well with the findings of pharmacological experiments in which a high content of glutamate has been found not only in the dorsal roots and dorsal root ganglia, but also in the dorsal column nuclei[2]. Furthermore, the glutamate content in the cuneate nucleus of the rat has also been shown to increase when the dorsal column fibers were stimulated[8]. In contrast, Sims et al.[9], in their histochemical study of succinic semialdehyde dehydrogenase in the rat brainstem, observed heavy staining for this enzyme, which destroys GABA, in the cuneate neuropil, but no staining at all of the cuneate neurons.

The identity of the stained neuronal perikarya observed in the present study is not known. Of particular interest is the observation of Galindo et al.[4] that hair, touch and proprioceptive cells in the cuneate nucleus were excited by iontophoretically-administered glutamate, the hair cells being the most responsive. It is not possible at this stage to correlate the latter observations with the present findings. However, several anatomical studies using horseradish peroxidase tracing techniques have led to the identification of the gracilo-thalamic projection cells in the cat[10] and the cuneothalamic projection cells in the rat[11]. In the latter study, Tan and Lieberman[11] were able to distinguish 2 classes of cuneothalamic projection cells in the rat; these cells were mostly medium-size round or oval cells which were within the range of diameters of the stained neurons of the same shape and size in the present study.

In the cat gracile nucleus, Berkley[10] has identified the neurons which projected to the inferior olivary nucleus as small, round cells of less than 10 µm diameter. The possibility that some of the stained neurons observed in the present study might be cuneothalamic and inferior olivary projection neurons will have to be verified.

The results of pharmacological experiments suggest that the medial lemniscal fibers are non-cholinergic and that the excitatory neurotransmitter substance is likely to be an amino-acid[12], and since the thalamus has been shown to have a high content of glutamate[13], and since the thalamic relay cells in the dorsal column nuclei are excited by iontophoretically-applied glutamate[14–18], it might be suspected that glutamate might be the excitatory transmitter substance in the lemniscal terminals in the somatosensory thalamus. Experiments are now underway to study the effect of an electrolytic lesion of the nucleus ventralis posterolateralis of the contralateral thalamus on the glutamic dehydrogenase activity in cuneate neurons of the cat. In addition, the effect on enzyme activity following rhizotomy of C5–C8 dorsal roots are also being studied.

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Functional properties of lyophilized hemoglobin in the presence of amino acids after 13 months of conservation

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Summary. Four lyophilisates of hemoglobin, each protected by an amino acid salt, were conserved for 13 months. The determinations carried out (oxyhemoglobin, methemoglobin, p50, Hill's number, and visible spectrum) demonstrated that the hemoglobin had retained its functional properties.

Oxyhemoglobin is very unstable in solution, even at low temperatures[1]. One would think that this molecule's conservation would be improved after lyophilization. However, 50% of the hemoglobin is oxidized to methemoglobin[2,3] during freeze-drying. This alteration can be prevented by the previous admixture of a number of very different substances: carbohydrates (glucose, ...), amine buffers and macromolecules[4]. However, recent evidence has shown that