Electron microscopic analysis of post-embedding immunogold labeling of GABA and Glutamate in cat trigeminal subnucleus caudalis after pulp extirpation of the mandibular molar teeth

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The glomerulus, a prominent structure of lamina II of the trigeminal subnucleus caudalis, may serve as a site of processing and modulation of nociceptive information in the brainstem trigeminal nucleus. This study was primarily a qualitative analysis of lesion-induced changes in glutamate(Glu) and gamma aminobutyric acid(GABA) immunoreactivity.

Four adult cats were used in this study. The subjects were premedicated with atropine and ketamine. Experimental animals were sacrificed at 15th day after pulp extirpation of the mandibular molar teeth. A 7mm section of brainstem which included subnucleus caudalis was blocked, and rinsed in 0.1 M phosphate buffer at 4℃. Vibratome sections of 100 μm in thickness were cut and processed by standard electron microscopic protocols. Sections were flat embedded between sheets of aclar film. Trigeminal subnucleus caudalis were selected, cut out with a razor blade, and remounted onto blocks for ultrathin sectioning. For double labeling of Glu and GABA, the grids were incubated with the primary antibody, rabbit anti-Glu, diluted at 1;10,000 and incubated with goat antirabbit IgG conjugated to 10 nm colloidal gold, diluted 1:20. Next the grids were incubated with the primary antibody, rabbit anti-GABA, diluted at 1;1000 and incubated with goat antirabbit IgG conjugated to 20 nm colloidal gold, diluted 1:20. The sections were examined in a JEOL 1200 EX II(Japan), electron microscope.

GABA immunoreactivity is observed in glomerular terminal profiles with pleomorphic vesicles. All the GABA-immunoreactive(IR) terminals exhibit normal ultrastructure, none of these GABA-IR terminals is seen to be degenerating. Glu immunoreactivity of central terminals is present in the acute stages of deafferentation and degeneration.

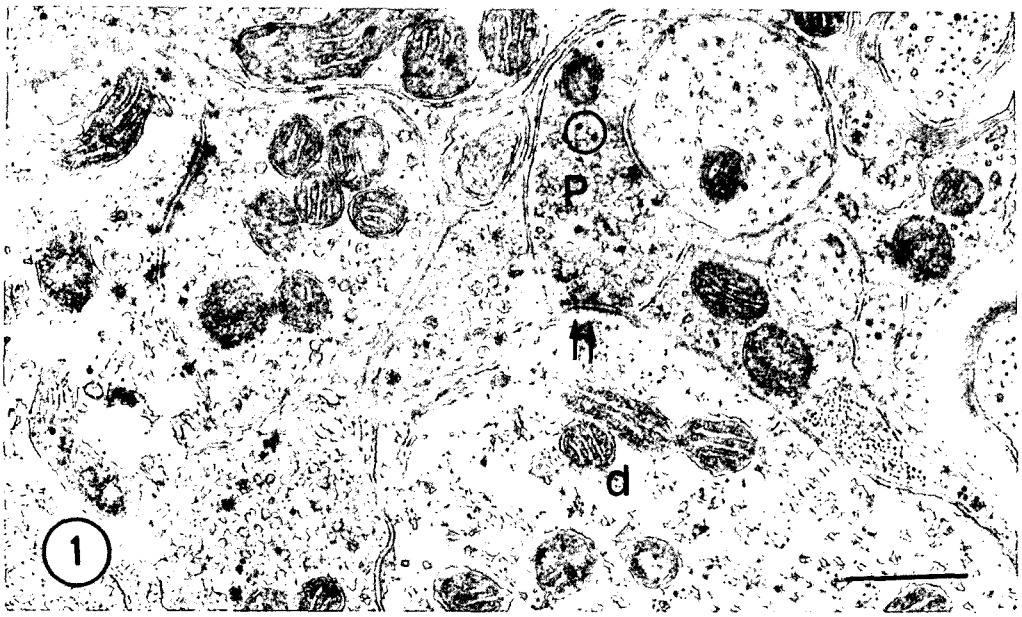

Fig. 1 Electron micrograph of the immunogold labeling of GABA and Glu in trigeminal subnucleus caudalis of normal cat. GABA immunoreactive terminal (P, 20nm gold particles = circle) exhibited symmetric (excitatory, double arrows) synaptic density. d, dendrite Scale bar = 0.5μm

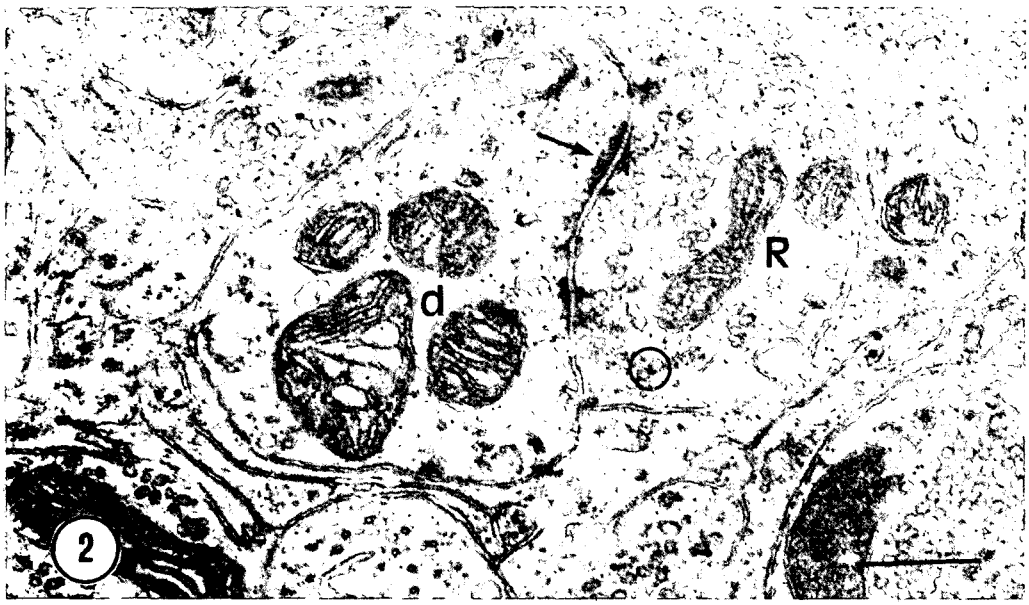

Fig. 2 Electron micrograph of the immunogold labeling of GABA and Glu in trigeminal subnucleus caudalis after pulp extirpation of the mandibular molar teeth. Glutamate immunoreactivity was observed in primary afferent terminal (R, 10nm gold particles = circle) which exhibited asymmetric (inhibitory, single arrow) synaptic density. Scale bar = 0.5μm