H^3-HRP Analysis of the Nerve Supply to Primate Teeth

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Sensory, sympathetic and parasympathetic ganglia located in the head and neck of rhesus monkeys were histologically examined after injection of H^3-HRP into the right mandibular premolars and molars. The results showed positive labeling of ganglionic cell bodies located in the ipsilateral trigeminal, superior cervical, and otic ganglia, plus the ipsilateral mesencephalic nucleus of the trigeminal nerve.

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Introduction.

The use of the tooth as a model system for studying the "trophic" action of the nervous system on the tissues and cells of the pulp and surrounding periodontium is predicated on a complete and definitive knowledge of the nerve supply to the structure(s) under investigation. In this regard, the literature provides equivocal evidence relative to the innervation of the teeth. The majority of the evidence regarding the innervation of the anterior teeth, incisors, and cuspids, seems to implicate transmedian innervation from the contralateral trigeminal ganglion.[1-7] The literature regarding the posterior teeth, bicuspids, and molars is confusing. Some reports suggest a collateral or accessory innervation from branches of the mandibular division of the trigeminal nerve other than the inferior alveolar nerve[8-13] and other studies suggest a nerve supply from other than the trigeminal complex, *i.e.*, the cervical plexus.[14-15]

Although some of the results from the above studies were derived from cadaver dissection or histological observation, the majority of the information was based on clinical studies in which the subject reported failure of anesthesia to the teeth after injection at the appropriate sites, and could be biased by the patient's threshold or tolerance to pain. Therefore, a more sensitive and reproducible system was needed to define the origin of the nerve supply to the dental pulp.

The use of horseradish peroxidase (HRP) for the intra-axonal retrograde tracing of central and peripheral neural pathways is now an established neuroanatomical technique.[16-18,20] Recently, Furstman *et al.*[16] have shown that horseradish peroxidase, after injection into rat tooth pulp, labeled the associated first order neuronal cell bodies located in the ipsilateral trigeminal ganglion. Arvidsson,[17] in a similar study on cats, unilaterally injected HRP into the upper and lower canines and found a discrete and somatotopic localization of the HRP in the ipsilateral trigeminal ganglion. Ellison and Clark[18] have demonstrated that HRP was transported to both parasympathetic and sympathetic cell bodies, although they did not use the tooth as their model. Based on these studies, Cox *et al.*[1] injected HRP into the pulps of the right maxillary and mandibular incisors, cuspids, bicuspids, and molars, and found HRP-positive cells located in both right and left trigeminal ganglia and the right superior cervical ganglion, and a group of four to ten cell bodies located in the ipsilateral pons. These results were of note for several reasons: The lack of sympathetic "cross-over" was of interest as was the presence of labeling in the pons. Also found was equivocal labeling of the ipsilateral otic ganglion in two of the five animals (unpublished results). The presence of HRP bilaterally distributed in the trigeminal ganglia was expected since the anterior teeth were injected and transmedian innervation of the central and lateral incisors was reported.[3-4] The present study was to further define and elucidate the primary neurons representing the sensory and auto-

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nomic innervation of primate tooth pulps using H^3-HRP and the techniques of quantitative autoradiography and electron microscopy.

Materials and methods.

Five adult rhesus monkeys were anesthetized with 1.0cc of ketamine hydrochloride and 1.5cc of sodium pentobarbital. Buccal Class V cavity preparations were cut in the mandibular first and second premolars and molars on the right side. Each of the four pulps was then entered by removing enamel and dentin to 0.5mm from the pulp with a #33 inverted cone bur. A small hole was made with a sterile explorer. Each of the pulps was then injected with one microliter of H^3-HRP (specific activity 50 Ci/mM).* The cavities were capped with calcium hydroxide compound and restored with silver amalgam to prevent leakage of the labeled HRP into the surrounding periodontium and oral mucosa. The animals were then returned to their cages and maintained on a stock diet. Seventy-two hours after injection of the isotope, the animals were anesthetized and sacrificed by intracardiac perfusion using Karnovsky's fixative.[19] Both ipsilateral and contralateral trigeminal, as well as geniculate, pterygopalatine, otic, and superior cervical ganglia were removed for evaluation. The brainstem and the submandibular ganglion were also removed. The tissues were post-fixed in Karnovsky's fixative overnight, washed in a Sorensen's buffer, dehydrated, embedded in methacrylate, cut serially at 4μm, and mounted on slides. The slides were then coated with Kodak NTB-2 emulsion, placed in light-tight boxes with a desiccant, and stored at 4°C. After an exposure period of twenty-one days, the slides were removed and developed in Kodak-D-19b for five minutes, dipped for 30 seconds in a distilled water stop bath, fixed for ten minutes in Kodak Rapid Fix, and washed for 30 minutes in three changes of distilled water. Every other slide was stained lightly with Harris' hematoxylin and coverslipped for brightfield microscopy. The remaining autoradiographs were left unstained and coverslipped for use with phase and Normarski differential interference microscopy. Reduced silver halide grains were counted over 500 labeled cell bodies with intact nucleus and nucleolus per ganglion per animal, except for the diffuse submandibular ganglia and brainstem, in which there was a lack of cells. Means and standard deviations were calculated for grain count data, and the Student's t test was used to evaluate statistical significance. The background was calculated, using a 10 x 10mm micrometer disk† for each slide.

Results.

Trigeminal ganglia. – Positive labeling was found in the right trigeminal ganglion as shown in Fig. 1, ipsilateral to the teeth injected. The labeled cells were located near the midline of the mandibular division of the ganglion with none at the periphery. The label was diffused throughout the cytoplasm of both large and small diameter ganglion cells. An average density of 9.42 grains per cell was found in the labeled cells of this ganglion. An electron microscopic autoradiograph of a right trigeminal ganglion cell is shown in Fig. 2. Grains can be seen randomly distributed throughout the cell. Some of the ganglion cells in the ipsilateral trigeminal ganglion were labeled more heavily than others (Fig. 3). Fiber tracts entering the ganglion could be seen with grains oriented in a linear array within the axon fibers. Some cells within the mandibular division of the trigeminal ganglion on the ipsilateral side were heavily labeled with grains while other cells in the immediate vicinity were negative (Fig. 3).

The left trigeminal ganglion cells did show an average of 1.69 grains scattered throughout the neuroplasm. This was considered to be slightly above background labeling, which was found to be the same as extracellular areas.

Superior cervical ganglia. – Positive labeling of H^3-HRP was seen in the ipsilateral superior cervical ganglion (SCG) cell bodies with a mean of 5.01 grains per cell.

The localization of grains in the perinuclear region of some cells in the trigeminal system was not observed in the superior cervical ganglion cell bodies (Fig. 4). Diffuse labeling within the cell bodies was found similar to some of the larger cells of the

*Amersham, Arlington Heights, IL

†Carl Zeiss, New York, NY

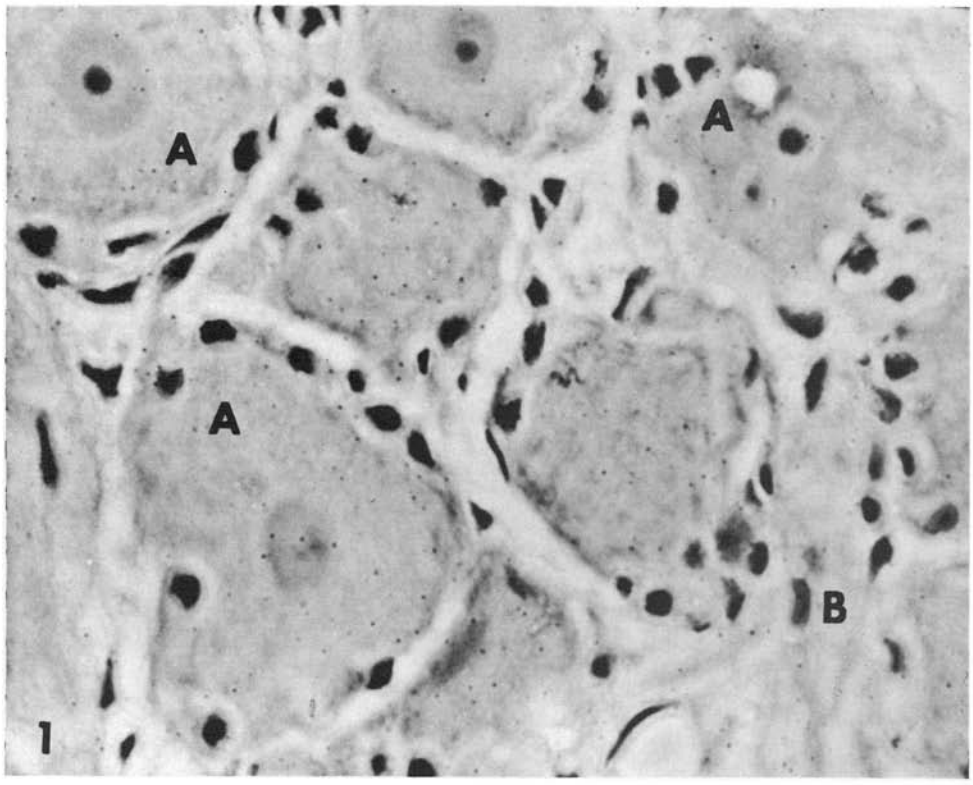

Fig. 1 – The trigeminal ganglion cells in this photomicrograph are ipsilateral to the injected teeth. Positive labeling is seen as silver grains located within the cell bodies (A), and in the axons coursing through the ganglion, lower right (B). H & E. x250.

trigeminal ganglion. These cells were localized in the anterior one-third of the SCG ganglion. No labeled H^3-HRP cell bodies were seen in the contralateral superior cervical ganglion (indicating no transmedian innervation of the cervical sympathetics to the injected posterior teeth).

Pterygopalatine ganglia. – The cell bodies located in the pterygopalatine ganglion, a parasympathetic ganglion associated with the Facial Nerve (VII), did not show uptake of the H^3-HRP on either the ipsilateral or contralateral side.

Submandibular ganglia. – The parasympathetic cell bodies of the submandibular ganglion, which are scattered throughout the stroma of the submandibular gland and usually located near ducts, did not show any uptake of the H^3-HRP anywhere in the substance of the gland on either the ipsilateral or contralateral side.

Otic ganglia. – The cell bodies located in the otic ganglion, associated with the Glossopharyngeal Nerve (IX), showed positive but diffuse uptake of tritiated label on the ipsilateral side in only one of the five animals. The H^3-HRP labeling was scattered throughout six of the cell bodies located in the basal region of the ganglion.

Geniculate ganglia. – The sensory cell bodies of the VII cranial nerve (Facial) located in the geniculate ganglion did not show any uptake of the H^3-HRP on either the ipsilateral or contralateral side.

Brainstem-mesencephalic nucleus of V. – Labeled cell bodies in the ipsilateral mesencephalic nucleus of V were a unique and unexpected finding, but were consistent in all animals examined using H^3-HRP. In some instances the labeling pattern was spread diffusely throughout the perikaryon, usually near the periphery of the cell. In other

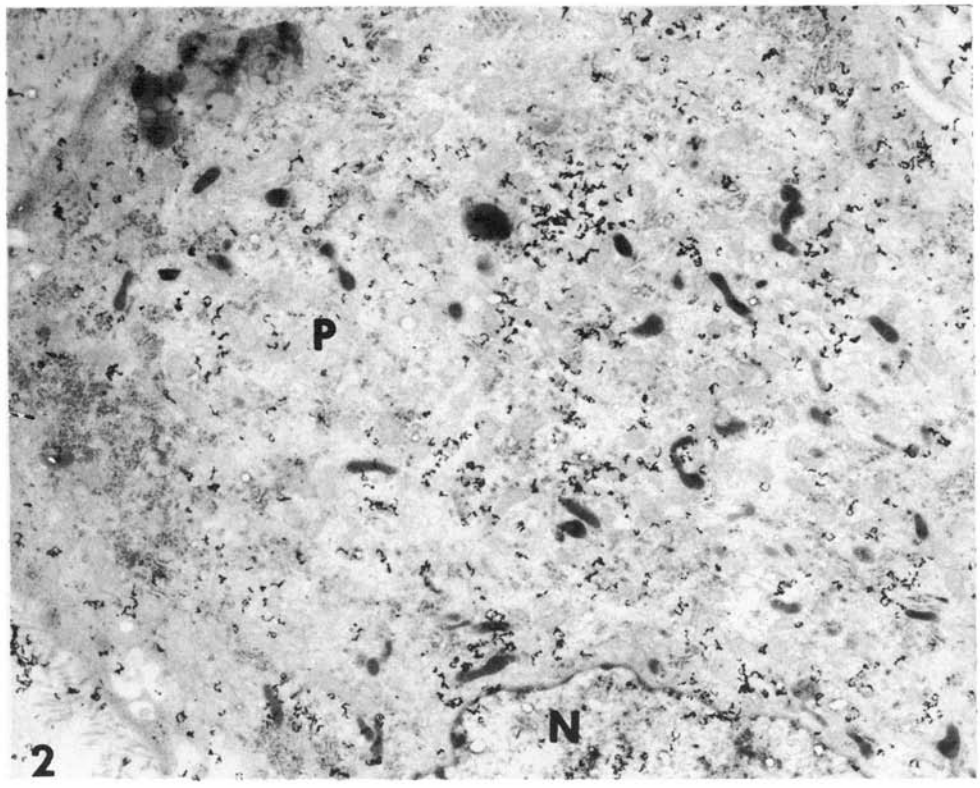

Fig. 2 – The HRP radiolabel in this electron microscopic autoradiograph is in the ipsilateral trigeminal ganglion. The grains do not appear consistently over specific organelles but are more randomly distributed throughout the perikaryon. Nucleus (N), Perikaryon (P). x4,960.

instances the labeling was found localized in specific areas of the perinuclear region as seen in Fig. 5. Six to ten positively labeled cell bodies were located in the mesencephalic nucleus of the trigeminal nerve in each animal, with an average of 5.38 grains per cell. The contralateral mesencephalic nucleus as well as other areas of the brainstem, including trigeminal sensory nuclei and motor nucleus, did not show any H^3-HRP label. The results of this study are summarized in Table 1.

The periapical region of each injected tooth was also examined microscopically for evidence of leakage of H^3-HRP from the pulp chamber into the surrounding periodontium. No evidence of leakage was found.

Discussion.

The original objective of the present study was to define the total nerve supply to the teeth by the use of the retrograde axonal transport of H^3-HRP. The results of this study generally corroborate our previous study of localization of HRP (H^3-HRP) in the right trigeminal ganglion, right superior cervical ganglion, as well as cell bodies in the pons, and suggest a possible role for the post-ganglionic parasympathetic neurons originating in the otic ganglion. This study provided additional information regarding the amount and location of H^3-HRP transported to these various ganglia. In the present study there was no contralateral labeling of the trigeminal ganglion. In a previous report, Cox *et al.*[1] injected the maxillary and mandibular incisors, cuspids, premolars, and molars ipsilaterally, and showed contralateral innervation from both the maxillary division and mandibular division of the trigeminal nerve. The labeled HRP in this

TABLE 1
MEAN ± STANDARD DEVIATION OF AUTORADIOGRAPHIC GRAIN COUNTS OVER SENSORY AND AUTONOMIC CELL BODIES IN THE PERIPHERAL AND C.N.S. SYSTEM AFTER H^3 – HRP ADMINISTRATION[1]

Ganglion / Animal	Trigeminal R	Trigeminal L	Geniculate R	Geniculate L	Superior Cervical R	Superior Cervical L	Pterygo-Palatine R	Pterygo-Palatine L	Otic R	Otic L	Submandibular R	Submandibular L	Trigeminal Mesencephalic Nucleus R	Trigeminal Mesencephalic Nucleus L
RH-1	9.87* ± 0.91	1.52 ± 0.06	0	0	4.61 ± 0.64	0	0	0	0	0	0	0	6.22 ± 0.12	0
RH-2	9.52* ± 0.34	1.37 ± 0.23	0	0	5.13 ± 0.59	0	0	0	0	0	0	0	4.13 ± 0.14	0
RH-3	11.45* ± 1.11	2.47 ± 0.41	0	0	4.72 ± 0.21	0	0	0	0	0	0	0	6.67 ± 0.23	0
RH-4	7.95* ± 0.96	1.41 ± 0.07	0	0	4.39 ± 0.24	0	0	0	0	0	0	0	5.32 ± 0.37	0
RH-5	8.31* ± 1.01	1.68 ± 0.23	0	0	6.21 ± 0.33	0	0	0	4.63 ± 0.18	0	0	0	4.54 ± 0.29	0

[1]Sample size for each mean and its standard deviation is not less then 500 cells except for the Brainstem and Submandibular Ganglion
*Mean values between the ipsilateral and contralateral cells are significantly different ($P<0.05$)

study was injected into the ipsilateral premolar and molar teeth and only found in significant amounts in the ipsilateral trigeminal ganglion. Therefore, transmedian innervation must occur only in the central and lateral incisors and/or the cuspids, and does not occur in the more posterior premolar and molars. Similar results have been reported by Anderson and Pearl[4] and suggested by Windle[21] and Brashear[22] who reported that up to 1/3 of the nerve fibers innervating cat pulps have their cell bodies in the contralateral ganglion as determined from electrophysiological and nerve degeneration techniques. The presence of a few grains in the contralateral trigeminal ganglia may be due to anterograde migration of the HRP to the cuspids and incisors. The possibility of collateral nerve supply to the posterior teeth, at least by a branch of the trigeminal system, cannot be ruled out at this time, however, and is presently under investigation.

Although the cell bodies located in the superior cervical ganglion on the ipsilateral side were positively labeled with H^3-HRP, the concentration of the tracer was approximately one-half of that found in the cell bodies located in the mandibular division of the trigeminal ganglion. Some of the possibilities which would explain these differences in the uptake of the labeled HRP are: 1. fewer sympathetic nerve endings in the pulp as compared to sensory; 2. possibly a slower rate of axoplasmic transport within the small fibers of the sympathetic nervous system compared to the trigeminal so that the H^3-HRP may not have reached the sympathetic ganglion by the time of sacrifice; and, 3.

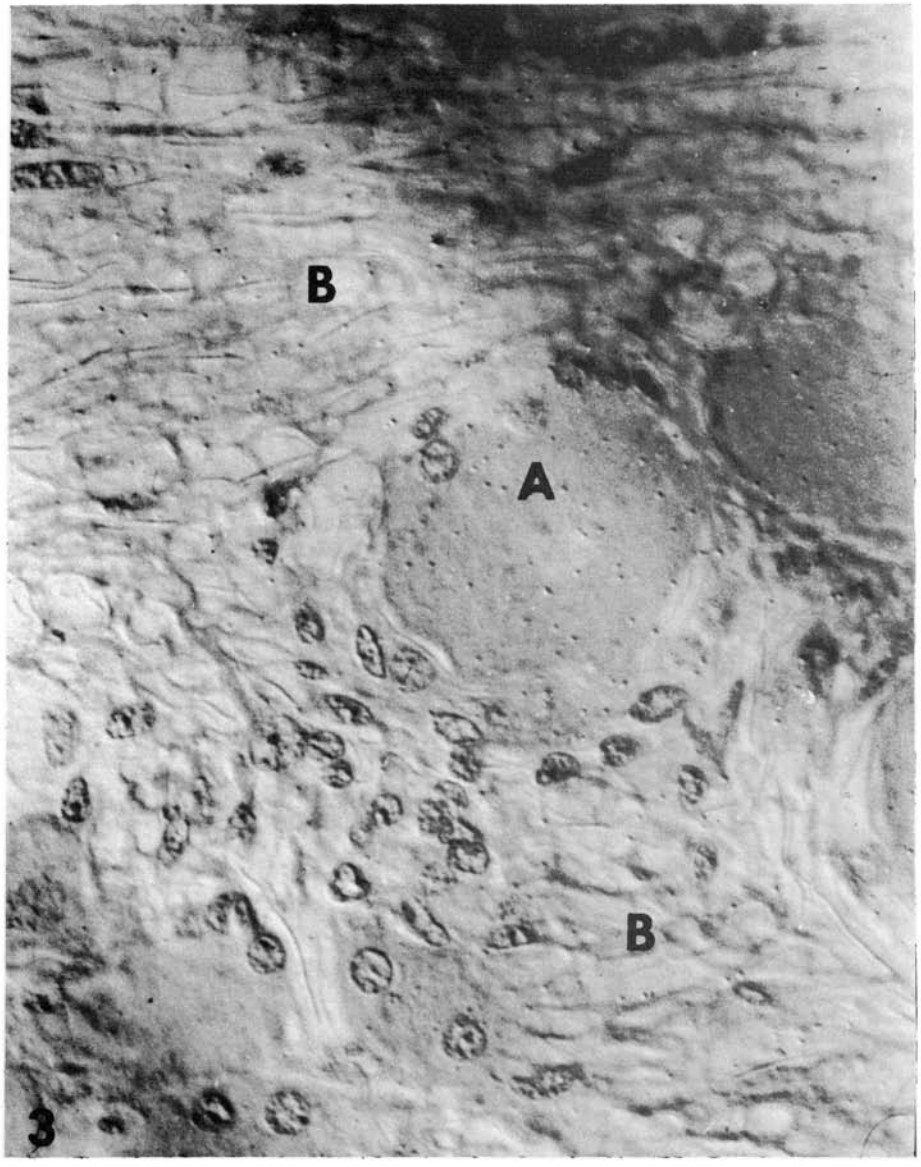

Fig. 3 – The ipsilateral trigeminal ganglion cell (A) in this light micrograph reveals labeling of H^3-HRP. The fiber tracts (B) also show labeling indicating transport of H^3-HRP from the teeth to other cells within the ganglion. Normarski differential interference contrast. H & E. x250.

less activity within the sympathetic system and therefore less uptake of the H^3-HRP at the nerve terminals.

The six to ten labeled cell bodies located in the ipsilateral mesencephalic nucleus of the trigeminal nerve were an unexpected and interesting finding. Piminidis and Hinds[23] have shown corpuscular nerve endings within tooth pulps of rats after administration of H^3-proline into the trigeminal ganglion.

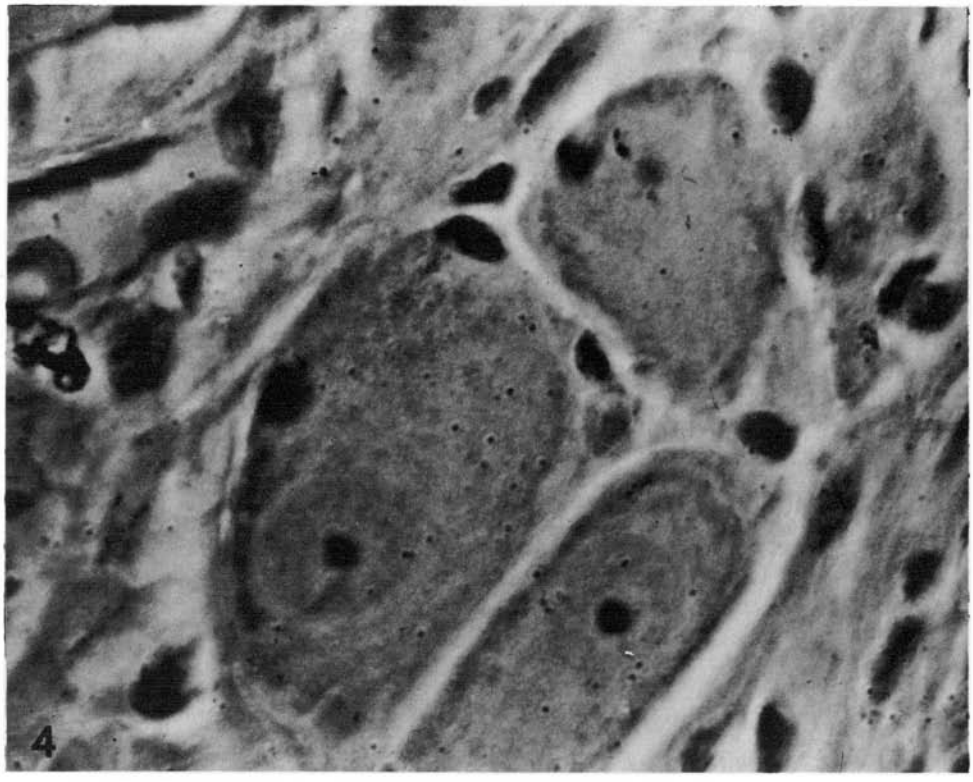

Fig. 4 – The positive labeled cells in this autoradiograph were located in the ipsilateral superior cervical ganglion. Note that the cells seem to incorporate less tritiated HRP than the cells in the ipsilateral trigeminal ganglion. H & E. x400.

They have suggested that these glomerular endings in the pulp were for touch and pressure and represented a specialized sensory modality other than pain. Seto[24] has described the presence of complex glomerular nerve endings in the human dental pulp but did not suggest a function. The fact that no radiolabel was found in the periapical region strengthens the belief that the transport of H^3-HRP is from pulpal nerves to the mesencephalic nucleus. This agrees with the findings of Piminidis and Hinds[23] and Seto[24] that there are primary afferents present in the pulp. Similar endings have been described and reported in the periodontal ligament by Harris and Griffin,[25] and Chiego *et al.*[26] and were interpreted as having a proprioceptive function.

The positive labeling of cells in the otic ganglion was also an unexpected finding. The presence of the tritiated HRP in only one of the animals would make it a questionable result; however, it was also found in two of the animals without the tritium-labeled HRP (unpublished results) and was present in approximately the same concentrations as seen in the superior cervical ganglion. It is possible that a longer exposure time is necessary to substantiate the presence of H^3-HRP in this ganglion. This labeling could also be the origin of the viable nerves found remaining in pulps after resection of the IAN and SCG.[27,29] The presence of post-ganglionic parasympathetic fibers in the dental pulp could be related to maintenance of homeostasis within the secretory and connective tissue elements of the pulp in response to trauma and in the modulation of reparative dentin.[28-29]

In summary, the use of H^3-HRP has been found to be an important neuro-anatomical technique for defining the complex innerva-

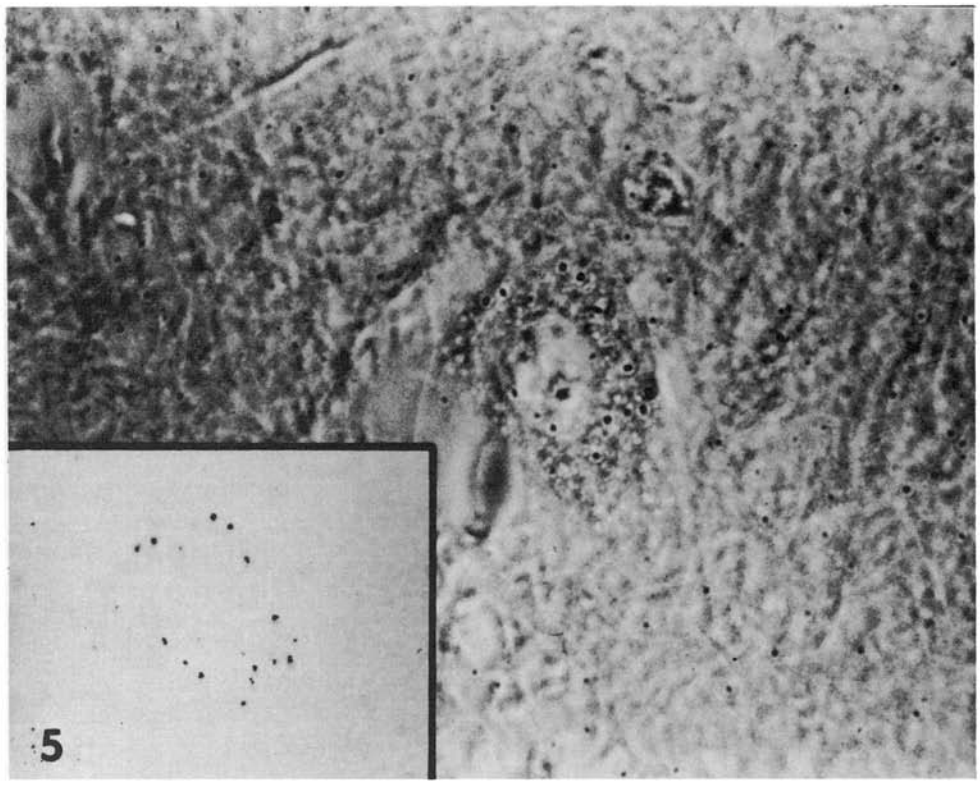

Fig. 5 – This autoradiograph shows one of the labeled cells in the ipsilateral mesencephalic nucleus. The label can be seen as the black silver grains surrounding the nucleus. The inset shows the same cell at a higher magnification focused only on the silver grains. Phase Contrast x 400.

tion of the primate teeth. The results showed positive labeling with H^3-HRP in the ipsilateral trigeminal ganglion and the ipsilateral superior cervical ganglion and significant quantities of H^3-HRP incorporated into the cell bodies within the ipsilateral mesencephalic nucleus of V. The contralateral trigeminal, otic, and superior cervical ganglia, as well as the contralateral mesencephalic nucleus of V did not show uptake of the tritiated label. The pterygopalatine, submandibular, and the geniculate ganglia did not show uptake of the H^3-HRP on either the ipsilateral or contralateral side. Studies are now in progress using a combination of neurophysiological recording techniques and stereotaxic injections of H^3-leucine into the trigeminal ganglion and mesencephalic nucleus to further define the neural pathways involved in the dentoalveolar complex.

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