Cells of Origin and Terminations of the Trigeminotectal Projection in the cat as Demonstrated with the Horseradish Peroxidase and Autoradiographic Methods

By

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—Received for Publication, August 14, 1981—

Key words: Trigeminotectal projection, Collicular patch, HRP, Autoradiography, Cat.

Summary: Organization patterns of the trigeminotectal projection in the cat were studied with the horseradish peroxidase (HRP) and autoradiographic techniques.

The projection was purely contralateral and most (68%) of the trigeminotectal cells were located in the oral nucleus, particularly in the rostral and ventral parts. Labeled cells in the oral nucleus had somal diameters of 22.8±7.4 μm (mean±SD) and were of the ovoid, fusiform, or triangular type. Other trigeminotectal neurons occurred in the principal (7%), interpolar (13%) and caudal (12%) nuclei. On the other hand, many trigeminothalamic neurons occurred contralaterally in the principal nucleus, most of which were small sized (15.4±4.0 μm) with round somata. Trigeminothalamic neurons were found bilaterally and those in the oral nucleus were somewhat larger (33.2±3.1 μm) and of the multipolar type.

After injection of tritiated leucine into the rostral and ventral parts of the oral nucleus, silver grains of the labeled trigeminotectal fibers were found contralaterally, terminating in the intermediate gray layer of the superior colliculus. The terminals were seen to form columnar patches, 100–500 μm wide, separated at 100–300 μm intervals. The discontinuous patches of terminals of other collicular afferents than the trigeminotectal projection were discussed with a comment on their morphological features that have been shown by other investigators.

By means of retrograde axonal transport of horseradish peroxidase (HRP), the distribution of trigeminotectal neurons of the cat has been studied in some detail (Baleydier and Mauguiere, 1978; Nagata and Kruger, 1979; Ogasawara, 1981). These studies have indicated that the intermediate and deep layers of the entire superior colliculus (SC) receive fibers from the contralateral sensory trigeminal nuclei, and that most of the trigeminal cells are located in the rostral and ventral parts of the oral nucleus. Cells of origin of other trigeminal neurons projecting to

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the thalamus (Karamanlidis et al., 1978; Fukushima and Kerr, 1979; Burton and Craig, 1979), cerebellum (Ikeda, 1979; Somana et al., 1980; Saigal et al., 1980), and spinal cord (Burton and Loewy, 1977; Matsushita et al., 1981) have also been demonstrated with the HRP method.

Electrophysiological studies (Stein and Arigbede, 1972; Stein et al., 1976) have shown that superficial layers of the SC are exclusively of a visual nature, while deep layers are predominantly non-visual. Intermediate layers are related to both the visual, auditory and somatic sensory functions. It can be assumed that impulses from various sources reach the SC and then are transferred to the collicular neurons projecting, for example, to the pontine nuclei (Hashikawa and Kawamura, 1977), reticular formation (Kawamura and Hashikawa, 1978), spinal cord (Kuypers and Maisky, 1975), and thalamic nuclei (Kawamura and Kobayashi, 1975; Raczkowski and Diamond, 1978). These neurons have recently been identified in the intermediate and deep layers with the HRP method.

The SC receives fibers from various sources (for references, see Edwards et al., 1979; Wurtz and Albano, 1980). Some of the collicular afferents have been demonstrated with the autoradiographic method to form a patchy pattern as is the case of the trigeminotectal terminals (Tashiro et al., 1980; Huerta et al., 1981). The present knowledge of the trigeminotectal projection, however, is relatively limited. In this study we aimed to obtain more detailed data to show exact sites of the origins and terminations of this projection, using retrograde (HRP) and anterograde (tritiated leucine) tracers.

**Materials and Methods**

Fourteen adult cats, weighting 2.3–4.2 kg, were used in this investigation. Thirteen of them received injections of 0.1–0.5 μl of a 50% (wt/vol) aqueous HRP (TOYOBO, Grade Ic) in the SC, thalamus, cervical spinal cord, and mesencephalic central gray matter. Another animal received a 0.5 μl of L-[4, 5-3H(N)] leucine (New England Nuclear, specific activity 45.7 Ci/m Mole) in the trigeminal nucleus. All animals were operated on under Nembutal anesthesia, and the injections were performed by means of a 25 gauge needle attached to a 1.0 μl Hamilton syringe.

After 41–77 hours of the HRP injections, the animals were deeply anesthetized and perfused through the heart with physiological saline followed by a mixture of 0.4% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate solution (pH 7.4). Serial frozen sections of 50 μm were cut horizontally (in cats KEN 127 R and 128 R) or transversely (in 12 cats) from the pons and medulla oblongata, and the midbrain was cut transversely in all animals. Every 5th horizontal section from the 2 cats (KEN 127 R and KEN 128 R) was treated with tetramethyl benzidine (TMB) according to the procedure of Mesulum (1978), and every 5th transverse section from the other animals was treated with diaminobenzidine (DAB) according to the methods described by Graham and Karnovsky (1966) and Streit and Reubi (1977).

In one cat, a 0.5 μl of a solution of tritiated leucine, dissolved in saline to give a concentration of 20 μCi/μl, was stereotactically injected into the rostral part of the right oral nucleus. The animal survived for 91 hours and was perfused, under anesthesia, with 0.9% saline followed by 10% formalin solution. The brain was dissected out from the skull and stored for 7 weeks in 10% formalin for further fixation. The brainstem was cut transversely on a freezing microtome at 25 μm. Every 10th section was dipped in Kodak NTB-2 emulsion,
and was kept in a light-tight box for 3 weeks. They were developed in Kodak D-19.

All sections, both HRP and autoradiography, were examined under the microscope with bright- and dark-field illuminations. The occurrence of HRP-labeled cells in the sensory trigeminal nuclei and injection sites in the SC, thalamus, and spinal cord was entered in drawings of sections made with the aid of a projection apparatus. Areas heavily stained with HRP were indicated in black and those of lighter staining were hatched. On the other hand, camera lucida drawings (Fig. 3) of labeled trigeminotectal fibers in the autoradiographs were made of serial sections from the SC.

Results

Trigeminotectal projections

As a representative case, the distribution and morphological features (shapes and sizes) of trigeminotectal cells were examined in cat KEN 128 R after injection of HRP (0.2 µl survival time 48 h, Fig. 2) in the intermediate and deep layers of the central and lateral parts of the right SC1). Labeled cells were found contralaterally throughout the sensory trigeminal nuclei2). The motor and mesencephalic trigeminal nuclei were devoid of labeled cells. The total number of the labeled cells in the entire sensory trigeminal nuclei was 407, 68% of which were found in the oral nucleus, particularly in its rostral and ventral parts (Figs. 1i and 2C). A smaller number of labeled cells were found in the principal (7%), interpolar (13%) and caudal (12%) nuclei. In the caudal nucleus, labeled cells were located in the ventral part of the magnocellular subdivision.

Somal diameters of the labeled cells in the entire sensory trigeminal nuclei were 11-55 µm (mean 22.6±6.7 µm, SD). Most of them were medium-sized (20-30 µm). Labeled cells in the oral nucleus, a total of 275, had diameters of 22.8±7.4 µm of the ovoid, fusiform, or triangular type (Fig. 1 b). Of the large (more than 40 µm) labeled cells of the multipolar type in the trigeminal complex, most (10 out of 13, indicated by squares in Fig. 2C) were located in the oral nucleus. The number of labeled cells in the principal nucleus was 27, and the majority were medium-sized (23.6±4.2 µm) with oval or round somata (Fig. 1 a). Diameter histograms of the labeled cells occurring in the principal and oral nuclei are shown in Fig. 2B. Mean diameters of labeled cells in the interpolar nucleus were 21.3±5.3 µm and no large cells were labeled in the nucleus. Most of them had round or ovoid cell bodies (Fig. 1 c). Labeled cells in the caudal nucleus had diameters of 24.3±6.8 µm of the round type, and a few large cells (Fig. 1 d) were also labeled in the magnocellular subdivision.

In addition to the heavy staining of the SC, a small lateral part of the mesencephalic central gray was lightly stained in this animal. As a control case, therefore, HRP was injected in the lateral part of the central gray matter in cat KEN 101 R (0.1 µl, 77 h, not illustrated). The stained area was virtually confined to the central gray matter and only five cells were labeled throughout the sensory trigeminal nuclei.

Tritiated leucine (10 µCi, 0.5 µl) was injected into the rostral part of the oral

1) The distribution of labeled trigeminotectal neurons after injections of HRP into various parts of the SC in other 9 animals has been described previously (Ogasawara, 1981).

2) The subdivision of the trigeminal sensory nuclei referred to in this study is based on the description given by Brodal et al. (1956) and Taber (1961).
trigeminal nucleus in *cat KH 98 R* (sacri-
fied after 91 h, Figs. 3 and 4). The rostral and ventral parts of the oral nu-
cleus were labeled. A number of labeled fibers were traced ventromedially from the oral nucleus to enter the contralat-
eral medial lemniscus. At the mesence-
phalic level, they were seen to ascend along the dorsal border of the medial lemniscus ("trigeminal lemniscus"). The labeled fibers ran dorsally to enter the SC and for the most part terminated in the superfi-
cial half of the intermediate layer of the colliculus, forming several discon-
tinuous patches (Fig. 4). Distribution of the patchy terminals is shown in the diagrams of Fig. 3. The labels, that represent trigeminotectal terminals, had a width of 100-500 μm with a non-labeled interval of 100-300 μm wide. The density and the width of the labeling varied throughout the SC. Most of the patches in the rostral part of the SC had apparently uniform widths of about 200 μm with heavy labeleng, whereas those in the caudal part of the SC appeared to be less discontinuous and had patches of varying widths.

**Trigeminothalamic cells**

After injection of HRP in the postero-
medial ventral nucleus (VPM) in *cat KEN 127 R* (0.3 μl, 41 h, not illus-
trated), labeled cells were found bilaterally in the sensory trigeminal nuclei with a contralateral pre-
ponderance. In the contralateral principal nucleus, many neurons were heavily la-
beled in the ventral part. Most (85%) of the labeled cells were small-sized (10-20 μm) with round somata (Fig. 1 e). Others were medium-sized (20-30 μm), and no large cells (more than 40 μm) were labeled. In the dorsal part of the ipsilateral principal nucleus, some cells were also labeled. Labeling of the cells in the oral nucleus was predominantly contralateral, while only a few cells were labeled on the ipsi-
lateral side. Most of the neurons were 15-25 μm in diameter with round or ovoid cell bodies (Fig. 1 f). Somal diameters of 304 labeled cells in the principal and oral nuclei were 15.0±4.0 μm (mean ±SD, see Fig. 2 B).

In the interpolar nucleus, many cells of various sizes were labeled contralaterally. In the caudal nucleus, some labeled cells, mostly medium-sized (15-25 μm), were found bilaterally in the marginal layer of the dorsal two-thirds of the nucleus.

**Trigeminospinal cells**

In *cat KEN 64 R* (0.5 μl, 49 h, not illu-
strated), HRP was injected in the upper cervical segment (C 2) on the right side. In addition to the heavy labeling of the injected area, spreading of the enzyme to the left side was noted. Labeled cells were found in the trigeminal nuclear complex, mostly located in the left oral nucleus. Various sizes (16-55 μm in diameter) of labeled cells were found in the trigeminal nuclei at levels from the facial nucleus to the inferior olive. Large mul-
tipolar cells (Fig. 1 g and h) were fre-
quently labeled. Mean diameters of tri-
geminospinal neurons were 33.2±3.1 μm (SD) and 25% of them were large (more than 40 μm) cells as shown in Fig. 2 B.

**Discussion**

Locations and morphological features of trigeminotetral neurons with reference to those of other trigeminal neurons

In agreement with recent HRP studies (Baleydierr and Maguire, 1978; Edwards et al., 1979; Ogasawara, 1981), the present study indicates that the intermediate and deep layers of the SC receive fibers from the contralateral trigeminal nuclei, and that many trigeminotectal neurons (68% of the total number of the labeled cells) are located in the oral nucleus, particularly in the rostral and ventral parts (Figs. 1 i
and 2 C). Labeled cells are also found in the principal (7% of the total number) and interpolar (13%) nuclei as well as in the magnocellular subdivision of the caudal nucleus (12%). In addition, many labeled cells are found in the caudal part of the oral nucleus, particularly in the ventrolateral part, which has not been described previously.

Of all trigeminotectal neurons (cf. cat KEN 128 R), 75% are located in the principal (7%) and oral (68%) nuclei, including large (40-60 μm) multipolar cells in the rostral pole of the oral nucleus (see Brodal et al., 1956; Eisenman et al., 1963). On the other hand, trigeminothamic (cf. cat KEN 127 R) and trigeminospinal (cf. cat KEN 64 R) neurons are also found in the principal nucleus as well as in the rostral part of the oral nucleus. In Fig. 2 B, the size distribution of somal diameters of the labeled cells in the principal and oral nuclei is presented to show the difference in the three kinds of trigeminal neurons. It is evident that mean diameters of trigeminothalamic neurons (15.0±4.0 μm) are much smaller than those of trigeminospinal (33.2±3.1 μm) and trigeminotectal (23.2±5.8 μm) neurons.

Projections from the sensory trigeminal nuclei to the thalamus (Karamanlidis et al., 1978; Fukushima and Kerr, 1979; Burton and Craig, 1979; Burton et al., 1979), spinal cord (Burton and Loewy, 1977; ten Donkelaar, H. J., 1978; Matsushita et al., 1981), and cerebellar cortex (Ikeda, 1979; Somana et al., 1980; Saigel et al., 1980) have recently been studied in several animals with the HRR method. Patterns of termination of trigeminotectal fibers compared with other tectal afferents

Our autoradiographic findings of discontinuous patches of termination of trigeminotectal fibers in the intermediate layer of the contralateral SC are largely in agreement with those obtained recently by Tashiro et al. (1980) and Huerta et al. (1981). The terminals are found in our experiment mainly in the superficial half of the intermediate gray layer and several

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(four to seven) patches of 100-500 μm wide with non-terminating zones of 100-300 μm are shown. Further, the present study clearly demonstrates that the patches occur in the entire colliculus and that they appear to constitute several longitudinal columns (Fig 3). Although some patches in Tashiro et al.'s sections (1980, cf. their Fig. 1) appeared in the optic layer in the caudomedial part of the SC, those in our materials are located only in the intermediate gray layer (Figs. 3 and 4). Concerning the labelings of patch-like terminals in the intermediate gray layer of the SC, those appearing in the rostral part are more uniform both in size and distribution than those seen in the caudal part (see Fig. 3 B). It may be mentioned, however, that the patches of label were not revealed in the most caudal part of the SC in Huerta et al.'s (1981) materials.

Patch-like patterns of the trigemino-tectal terminals within the intermediate layer are similar to those of fibers from the cat substantia nigra (300-500 μm wide, unsharply separated at roughly 100-300 μm intervals, shown by Graybiel, 1975; 1978), the monkey frontal eye field (300-500 μm wide with a gap of similar width, shown by Künzle et al., 1976), and the rat somatosensory cortex (200 μm wide with a gap of less than 200 μm, shown by Wise and Jones, 1977). According to Wise and Jones (1977), there is an organized somatotopic pattern within the collicular projection from the somatosensory cortex of the rat. For example, the face-head region is represented in the rostral part of the SC, while the limb region is represented in the caudalateral part. Retinotectal fibers, on the other hand, have been shown to have a visuotopic pattern (e.g., Apter, 1945; Feldon et al., 1970): the central receptive field is represented in the rostral part of the SC, and the peripheral in the caudal. The fibers terminate in the superficial gray layer of the SC, forming patch-like patterns of 200 μm wide with a gap of 200 μm (Graybiel, 1975; see also Hubel et al., 1975; Harting and Guillery, 1976; Weber et al., 1978). All of these terminals in the SC have the labeling of discontinuous patches of approximately the same width.

These morphological findings are difficult to evaluate with reference to the functional aspects of the SC. However, it may be of relevance to mention the visuotopic-somatotopic correlation proposed by Stein and his coworkers (Stein and Arigbede, 1972; Stein et al., 1974, 1976; Stein, 1978). With the microelectrode technique, they found that the visual and somatic representations in the superficial and intermediate gray layers, respectively, are likely to be in register despite their partial laminar segregation. The central visual representation overlaps the somatic representation of the face, and the peripheral representation overlaps the body representation. Although morphological basis for this has not yet been available, vertical columnar connections between the two gray layers in the SC are likely to be present.

Reference

4) Brodal, A., T. Szabo & A. Torvik: Corticofugal fibers to sensory trigeminal
26) Matsushita, M., N. Okado, M. Ikeda &


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Al</td>
<td>Stratum album intermedium</td>
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<tr>
<td>B. p.</td>
<td>Brachium pontis</td>
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<tr>
<td>F. l. m.</td>
<td>Fasciculus longitudinalis medialis</td>
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<tr>
<td>GI</td>
<td>Stratum griseum intermedium</td>
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<td>GS</td>
<td>Stratum griseum superficiale</td>
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<tr>
<td>N. c.</td>
<td>Cochlear nuclei</td>
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<tr>
<td>N. f. c.</td>
<td>Nucleus cuneatus</td>
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<tr>
<td>N. f. g.</td>
<td>Nucleus gracilis</td>
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<tr>
<td>N. m. X</td>
<td>Dorsal motor (parasympathetic) nucleus of vagus</td>
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<tr>
<td>N. V, N. VII</td>
<td>Root fibers of cranial nerves</td>
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<td>Nucl. caud. V</td>
<td>Nucleus caudalis of spinal Vth nucleus</td>
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<tr>
<td>Nucl. i. p. V</td>
<td>Nucleus interpolaris of spinal Vth nucleus</td>
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<tr>
<td>Nucl. o. V</td>
<td>Nucleus oralis of spinal Vth nucleus</td>
</tr>
<tr>
<td>Nucl. pr. V</td>
<td>Nucleus principalis of trigeminal nerve</td>
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<tr>
<td>O</td>
<td>Stratum opticum</td>
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<tr>
<td>Ol. s.</td>
<td>Superior olive</td>
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<tr>
<td>Tr. sp. V</td>
<td>Spinal tract of trigeminal nerve</td>
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<tr>
<td>Z</td>
<td>Stratum zonale</td>
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<tr>
<td>III, V, VI, XII</td>
<td>Motor nuclei of cranial nerves</td>
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Explanation of Figures

Plate I

Fig. 1. Photomicrographs showing labeled cells in the sensory trigeminal nuclei after injections of HRP into the superior colliculus (a–d, bright-field illumination), ventrobasal thalamus (e and f, bright-field illumination), and upper cervical cord (g and h, dark-field illumination). Magnification of these pictures are the same, and scale bar in “a” represents 30 μm. 

a: Photomicrograph of a trigeminotectal cell from the ventral part of the principal nucleus.
b: Photomicrograph of two trigeminotectal cells from the medioventral part of the rostral oral nucleus.
c: Photomicrograph of two trigeminotectal cells from the interpolar nucleus.
d: Photomicrograph of a trigeminotectal cell from the magnocellular layer of the caudal nucleus.
e: Photomicrograph of trigeminothalamic cells from the principal nucleus.
f: Photomicrograph of trigeminothalamic cells from the rostral pole of the oral nucleus.
g and h: Photomicrographs of trigeminospinal cells from the oral nucleus.
i: Photomicrograph of trigeminotectal cells from the ventral part of the oral nucleus indicated in Fig. 2C. Bright-field illumination. Scale bar 200 μm. Arrows indicate giant cells (about 60 μm in diameter) located in the rostral pole of the oral nucleus.
Plate 1

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Plate II

Fig. 2. Diagram showing the findings in one animal (KEN 128R) with an injection of HRP (50%, 0.2 μl) in the right superior colliculus. The extent of the injected enzyme was plotted on the surface of the superior colliculus, and shown as seen in two transverse sections through the colliculus (A, levels indicated in a diagram of the colliculus above). Black denotes heavy staining of HRP, hatching varying degrees of lighter staining. To the right (C) drawings of six horizontal sections through the trigeminal nuclear complex in the same animal (from ventral to dorsal, numbered 17-23) showing the distribution of labeled cells in the brainstem on the left side. Dots, triangles and squares indicate small, medium-sized and large neurons, respectively. Note some difference in the size distribution of labeled trigemino-tectal neurons as plotted in a diagram (B, indicated by thick lines, cf. cat KEN 128R) when compared with that of trigemino-thalamic (thin lines, cf. cat KEN 127R) and trigemino-spinal (broken lines, cf. cat KEN 64R) neurons.
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Plate III

Fig. 3. Diagram showing the findings in one animal (KH 98R) with an injection of tritiated leucine (10 μCi, 0.5 μl) in the rostral and ventral parts of the right oral nucleus (A, level indicated in an inset figure). Below and to the right (C) camera lucida drawings of ten transverse sections through the colliculus in the same animal (from rostral to caudal, numbered 10-25) showing the distribution of trigeminotectal terminals. Note patch-like labeling in the intermediate gray layer of the colliculus contralateral to the injection site. Overall distribution of labeling in the superior colliculus is illustrated in a dorsal-view reconstruction (in B). The locations of patch-like zones of labeling are shown by short lines.
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Plate IV

Fig. 4. Dark-field photomicrographs of two (a and b) transverse sections showing termina.
patches of the trigeminotectal labeling in the intermediate gray layer of the contralateral
colliculus indicated in Fig. 3. Scale bars 200 μm.
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