

# RECIPROCAL TOPOGRAPHY BETWEEN THE TOAD'S TECTUM AND NUCLEUS ISTHMI AND CELL CLASSIFICATION

WANG YINTING (王荫亭), YAN KUN (颜坤) AND WANG SHURONG (王书荣)

(*Institute of Biophysics, Academia Sinica, Beijing*)

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Our preceding papers<sup>[1-3]</sup> have shown that the nucleus isthmi (NI) is a secondary visual center in anuran amphibians. We, using HRP tracing and Golgi-Cox impregnation techniques, study the relationship between NI and the optic tectum (OT) of toads, morphology of reciprocally projecting cells, and connections of NI with its surrounding structures in this report.

## I. MATERIALS AND METHODS

Toads *Bufo bufo gargarizans* were used in this experiment. Surgical operations and stereotaxic fixation were described previously<sup>[2,3]</sup>. The OT superficial layers in 53 toads and NI in 13 were HRP-injected. One injection was given to each toad, and injection coordinates were different from animal to animal.

Beveled pipettes (10–30  $\mu\text{m}$ ) were tip-filled with 20% HRP solution (Sigma VI, 0.05 M Tris, pH 8.6). For iontophoresis positive pulses, duration 0.5 sec, 1 Hz, 2–3  $\mu\text{A}$ , were applied for 15–25 min. Negative retaining current was used to prevent HRP from leaking into electrode tracks.

After 2–8 days' survival at 16–20°C, animals were reanesthetized with ether and transcardiacally perfused with a physiological saline followed by 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were frozen-cut and demonstrated for HRP activity<sup>[4]</sup>.

Additional 11 brains were impregnated with Golgi-Cox method<sup>[5]</sup>, and celloidin-cut. All sections were counterstained with cresyl violet.

A camera lucida was used to draw HRP-injected sites, as well as locations and morphology of HRP-labeled and Golgi-Cox-stained cells.

## II. RESULTS

### 1. Reciprocal Topography Between OT and NI

After injecting HRP into the OT superficial layers, the labeled isthmie cells cluster, which correspond topographically to the injected sites: the medioventral NI projects ipsilaterally onto the rostral tectum, dorsal NI corresponds to caudodorsal

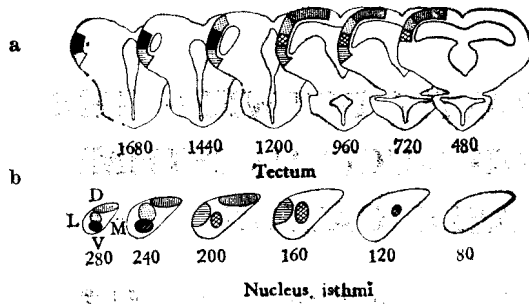


Fig. 1. Representations of tectum on the ipsilateral NI. Legends of isthmic injection area (B) are the same as those representing tectal labeled ones (A). Numerals indicate distance ( $\mu\text{m}$ ) from the caudal pole of the ipsilateral tectum. D, L, M, V signify dorsal, lateral, medial, ventral, respectively.

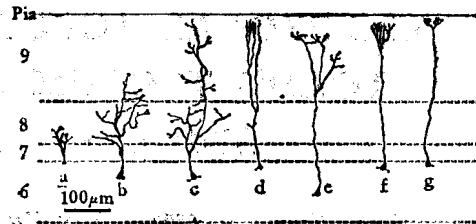


Fig. 2. Types of HRP-labeled tectal cell located in layer 6. Numerals indicate tectal lamination.

tectum, and middle NI is represented on the middle area of the dorsal tectum. In 5 cases, there were also HRP-labeled cells in the contralateral NI, however, they were faintly stained and a few in number.

Isthmic HRP injection labels only tectal cells ipsilaterally in a wedged section, which corresponds to the injected site topographically (Fig. 1): the lateral tectum projects onto NI ventrally and laterally, dorsolateral tectum corresponds to the NI central medulla, and caudodorsal tectum is represented on dorsal NI. It is obviously indicated that there is a reciprocal topography between OT and NI in toads.

## 2. Classification of HRP-labeled Tectal and Isthmic Cells

Ninety percent of labeled tectal cells are located in layer 6, and belong to pyramidal neurons, which may be classified into 3 types based on branching patterns of their apical dendrites: (i) those with dendritic shafts ascending radially, very close to the pia they end in small bushes (Fig. 2f, g); (ii) those whose apical dendrites arborize at different levels in a single layer (e); and (iii) those which distribute their dendritic branches in several layers (a—d). The majority of these cells possess 1—2 slender and short basal dendrites. However, we were unable to identify their axons.

The majority of isthmic cells have large perikarya, wide dendritic fields, and their dendrites trend to spread out dorsolaterally (Fig. 3). According to the number and distribution of their primary dendrites, these cells may be grouped into 3 types: (i) multipolar neurons having more than 3 primary dendrites (Fig. 3a), including those with radial dendrites (a1), those with dendrites spreading in the opposite directions (a2), or in a single direction (a3); (ii) bipolar cells having two primary dendrites (Fig. 3b), including those with dendrites in one direction (b1), or in the opposite directions (b2); and (iii) monopolar cells (Fig. 3c) including those without dendritic arborization (c1), those with finer branches on the tapered (c2) or basal

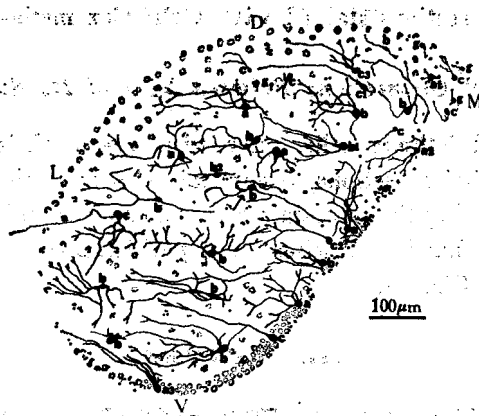


Fig. 3. Types of HRP-labeled isthmic neurons. Cell b pointed with arrow is enlarged in Fig. 4H-B. n—axon; D, L, M, V signify dorsal, lateral, medial, ventral, respectively.

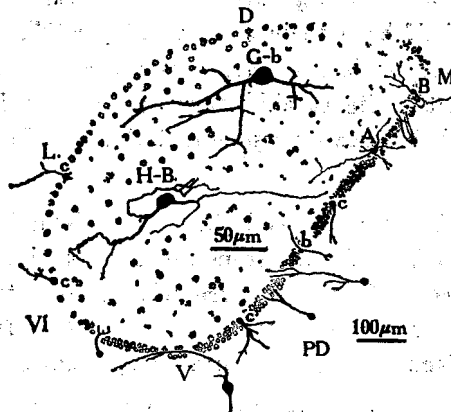


Fig. 4. Morphology of the trans-boundary cells and differences in appearance between HRP-labeled (capitals) and Golgi-Cox stained (littles) cells. Letters adjoining somata indicate the same classification as those in Fig. 3. Original locations of medullary cells H-B and G-b are similar, pointed with arrow in Fig. 3. VI—secondary visceral nucleus, PD—posterodorsal tegmental nucleus. D, L, M, V indicate dorsal, lateral, medial, ventral, respectively.

(c3) parts of the dendritic shaft.

### 3. Comparisons of HRP-labeling With Golgi-Cox Staining

HRP-labeled isthmic cells have smoother surface and some spines only on their finer dendritic branches, whereas Golgi-Cox stained cells have numerous spines on both dendritic shafts and finer branches. Fig. 4 shows difference between the two staining methods by taking two medullary cells as example.

In HRP-labeled materials, there is, in addition, a type of granular cell having smaller somata as well as very thin and short dendrites (Fig. 3g). Nevertheless, they

never appear in isthmic sections stained with Golgi-Cox method.

#### 4. Neuronal Connections Between NI and Its Surroundings

Of all HRP-labeled isthmic cells, only two spread their dendrites out of NI (Fig. 4A, B). In Golgi-Cox sections, 5 cortical cells have their dendrites in the peri-isthmic area, probably being the secondary visceral nucleus and posterodorsal tegmental nucleus. On the other hand, dendrites of 6 peri-isthmic neurons end within NI or pass through its cortex (Fig. 4).

### III. DISCUSSION

Our results show that there is reciprocal topography between the toad's OT and NI. This is in agreement with that obtained on frogs<sup>[6,7]</sup>.

In toads, tectal cells projecting to NI are mainly pyramidal neurons located in layer 6, and some multipolar cells in layers 2 and 4, but no labeled cells appear in layer 7. However, of tectal cells responding to electrical stimulation of the optic nerve of frogs, about one half have their somata in layer 7<sup>[8]</sup>. It seems likely that cells located in this layer do not project to NI, or our injections do not reach the specific projection area of these cells. Ito et al.<sup>[9]</sup> have shown that in fish, cells projecting to NI give out axons which bifurcate perpendicularly. We do not find this phenomena in toads.

This study indicates that none of cells in the peri-isthmic tegmentum projects to the superficial layers of OT, different from the results obtained on mammals<sup>[10,11]</sup>. Neuronal connections between NI and its surroundings seem to be very weak. These are consistent with our electrophysiological studies on NI of frogs<sup>[1,2]</sup> and toads<sup>[3]</sup>, i.e. NI is a well-demarcated visual center in anuran amphibians.

Differences in neuronal appearance between HRP-labeling and Golgi-Cox impregnation might be explained by electron-microscopic observations that HRP molecules are only restricted to the cytoplasmic vesicles<sup>[12]</sup>, whereas Golgi black precipitate can fill cells and their processes or selectively stay in extracellular spaces<sup>[3]</sup>.

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