

## Neuroanatomy and electrophysiology of the lacertilian nucleus isthmi

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Horseradish peroxidase (HRP) tracings showed a bidirectional connection between the optic tectum (OT) and the ipsilateral nucleus isthmi (NI) pars magnocellularis (Imc) in nocturnal *Gekko gekko* and diurnal *Shinisaurus crocodilurus*. We found that, in addition to a direct neuronal pathway, there is an indirect pathway from OT to Imc via the nucleus profundus mesencephali (NPM). The morphology of cells in OT, NPM and NI was studied. Visual units extracellularly recorded from NI were located within Imc based on cobalt sulphide markings. They responded to moving contrast targets, without reacting to tactile and auditory stimulation. HRP and Golgi–Cox studies showed that Imc is a nucleus independent of its parvocellular partner, Ipc.

Several neuroanatomical and electrophysiological studies on the nucleus isthmi (NI) have been made on teleosts<sup>7,20</sup>, anurans<sup>3,4,15–19</sup> and birds<sup>6,14</sup>; the parabigeminal nucleus, the mammalian analogue of NI, has also been investigated in both aspects<sup>2,9,13</sup>. However, this is not the case with reptiles, although they are the first terrestrial vertebrates and occupy a strategic position between amphibians on one hand and birds, not mammals on the other. To our knowledge, there have only been a few neuroanatomical papers on the reptilian NI<sup>1,12</sup>. Up to date, nothing is known about the electrophysiological properties of isthmic units in reptiles.

Our previous studies show that NI is a secondary visual center in anurans<sup>16–18</sup>. Thus, the lack of knowledge about the neuroanatomy and electrophysiology of the reptilian NI could hamper our understanding of the visual system of reptiles in particular and of vertebrates in general. Therefore, the present study on the lacertilian NI was carried out in order to reveal: (1) neuroanatomical relationship between optic tectum (OT) and NI; (2) morphology of cells in these structures; and (3) electrophysiological properties of isthmic units.

The experiments were performed on adult *Gekko gekko* with a body length of 11–14 cm (from the tip of the nose to the cloaca) and on *Shinisaurus crocodilu-*

*rus* 13–16 cm long, both of which were wild-caught from southern China. For anatomical investigations, HRP was injected into either NI or the superficial layers of OT. After two days survival, HRP activity was demonstrated following 'brown reaction' procedures<sup>5</sup>. Additional 5 gekkoes were sacrificed and their brains impregnated using the Golgi–Cox method<sup>11</sup> to analyze mesencephalic cytoarchitecture. For electrophysiological experiments, a minimal dose of gallamine triethiodide (4%) was injected intramuscularly. NI is a macroscopic oval swelling, located at the edge of the anterior medullary velum, immediately in front of the superficial origin of the trochlear (IV) nerve. Meninges overlying NI, or part of OT to be injected with HRP (Sigma VI), were removed. The animal was fixed in a stereotaxic apparatus, the center of a perimeter was aimed at the middle point of the interocular line. Presentation of visual, tactile and auditory stimulation has been described previously<sup>16–18</sup>. Electrophysiological recordings were made with micropipettes, 3–4  $\mu\text{m}$  tip diameter, containing 2.5 M NaCl and 50 mM  $\text{CoCl}_2$ . Recording sites in some lizards were marked with cobalt sulphide<sup>10</sup>.

Tectal HRP injections were made in 18 gekkoes and 3 shinisauruses. In every case, some cells in the ipsilateral Imc were labeled topographically. In gen-

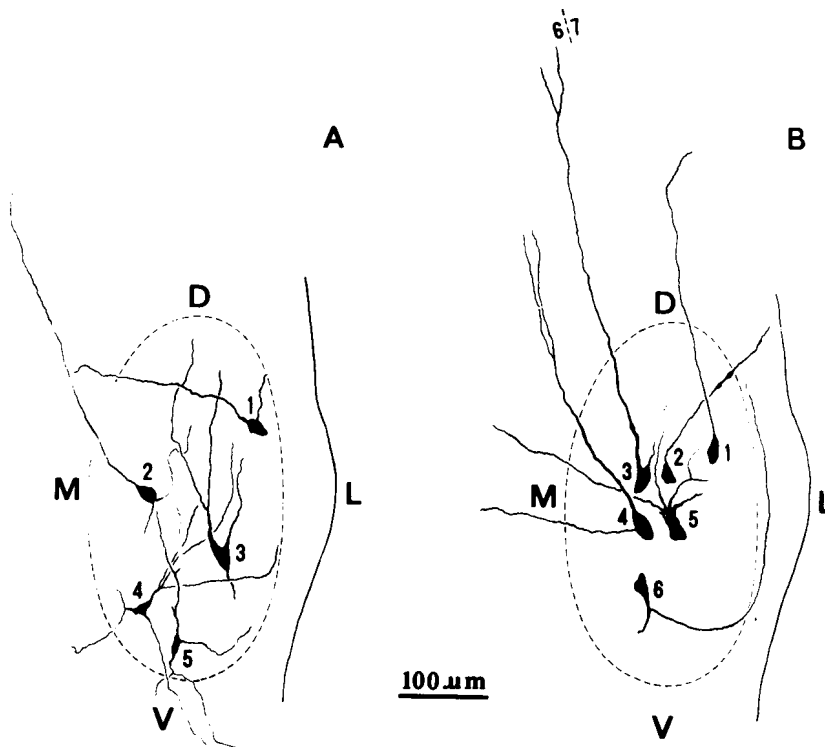
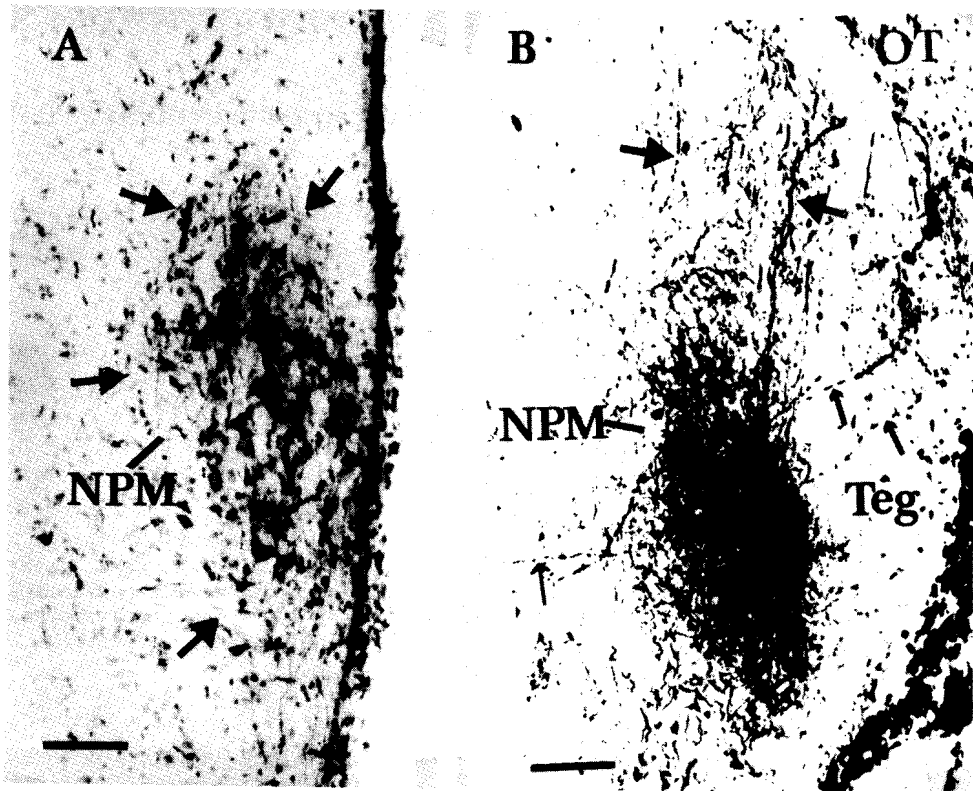


Fig. 1. Microphotographs showing NPM labeling after tectal (A) and isthmic (B) HRP injections (upper), and composite camera lucida drawings of NPM cells labeled following tectal (A) and isthmic (B) injections (lower). Arrowheads indicate dendrites traveling to tectum (OT); thin arrows, dendrites spreading out into tegmentum (Teg); thick arrows show NPM borders. Bars: 100  $\mu$ m. D,L,M,V signify dorsal, lateral, medial, ventral. 6,7, tectal layers.

eral, rostral injections resulted in medially labeled Imc, lateral injections in the central part, and caudal injections in the dorsal part of Imc. Labeled cells have piriform or polygonal somata with 1–3 primary dendrites, usually having 1–2 branches which bear a few varicosities occasionally. Isthmic cells labeled in *Shinisaurus* have smaller somata and thinner dendrites compared to those of *Gekko*. These cells were frequently located in an area of labeled fibers and granules, presumably being in an anterogradely labeled terminal field. There was only one case in which tectal injection led to labeling in bilateral Imc. Contralaterally labeled cells were faintly stained, with shorter processes. In any case, isthmic labeling was strictly limited within Imc. Golgi-Cox study showed no neuronal connections between Imc and Ipc. Dendrites of Imc cells located on the Imc-Ipc border spread out laterally.

Isthmic injections were made in 7 *Gekko* and 1 *Shinisaurus* specimens. Topographically labeled tectal cells were mainly located in layer 7, with a few scattered in layers 8–11. Cells in layer 7 usually have piriform or pyramidal somata, which radiate an apical dendrite pointing towards the pia and 1–2 slender and short basal dendrites. Their dendritic fields spread in columns; in some cells dendritic branches bear numerous varicosities, which often begin to appear in layer 9 and reach their greatest density in layers 11–12. Cells labeled in layers 8–11 are fusiform or polygonal in shape, having smaller dendritic fields. Golgi-Cox materials showed some horizontal cells in layer 7, which have larger polygonal or piriform somata, with a large proportion of the dendrite restricted to this layer. However, this kind of elements was never labeled by HRP injected into NI. Therefore, it is likely that they are horizontally connecting cells, at least not projecting to NI.

Following all isthmic injections, the ipsilateral NPM was also simultaneously labeled. Labeled cells possess polygonal or fusiform somata, whose thick dendrites frequently travel out of NPM borders, particularly to the tectum. Of 18 *Gekko* specimens injected tectally, the ipsilateral NPM of 7 animals was labeled simultaneously with the isthmus. Both Imc and NPM were labeled ipsilaterally after tectal injections in 1 out of 3 *Shinisaurus* animals. These NPM cells were generally more lightly stained and much fewer in number, compared to those labeled after

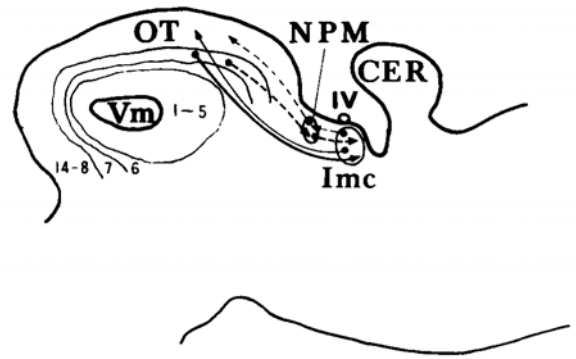


Fig. 2. Neuronal pathways connecting optic tectum (OT) and nucleus isthmi pars magnocellularis (Imc). Solid lines represent direct pathways; dashed lines indirect pathways via nucleus profundus mesencephali (NPM). Arrowheads denote axonal terminals; closed circles somata. CER, cerebellum; Vm, mesencephalic ventricle; 1–14, tectal lamination; IV, cross-section of the trochlear nerve.

isthmic injections. They have polygonal or fusiform somata, dendrites mostly restricted within NPM, some projecting into the adjacent tegmentum (Fig. 1). An NPM injection stereotaxically made in 1 *Gekko* labeled some cells in both OT and Imc ipsilaterally. However, these tectal cells might be those whose fibers participate in the tectobulbar pathway and terminate in NPM<sup>1</sup>, or pass through this nucleus in the tecto-isthmic tract<sup>12</sup>, whereas isthmic cells labeled following NPM injection might be those whose fibers pass through NPM to the tectum<sup>12</sup>. It appears that there exist at least two neuronal pathways from OT to Imc: a direct tecto-Imc and an indirect tecto-NPM-Imc pathway (Fig. 2).

Electrical responses to visual stimulation were recorded from 105 isthmic units in *Gekko* and 11 in *Shinisaurus*. Half of them were spontaneously active (0.3–5 spikes/s). About 50% of the units responded to a change in background illumination. It is noteworthy that unit GPO4 from *Gekko*, discharging briskly in the dark, was inhibited by the onset of background illumination; on the contrary, unit SPO6 from *Shinisaurus* was completely silent in the dark, but was activated by the onset of background illumination (Fig. 3A). According to Kayama et al<sup>8</sup>, these units are scotergic and photergic, respectively. The lacertilian isthmic units gave vigorous responses to contrast targets moving through their receptive fields (RF). About 35% of these units showed transient responses in a burst consisting of a few spikes only at

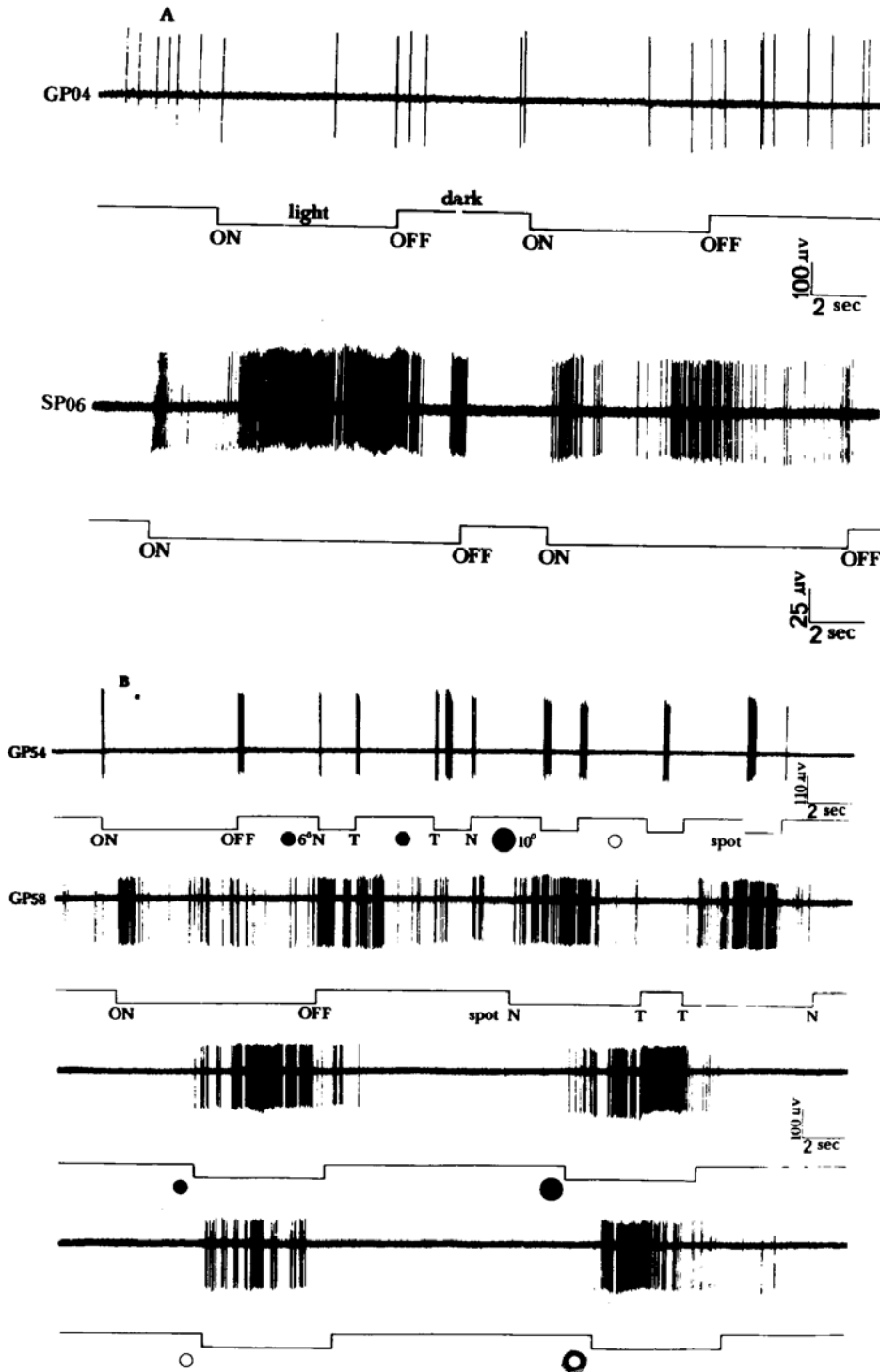


Fig. 3. Electrical responses of isthmic units to change in background illumination (A), and to light spot, black (●) and white (○) discs (B). GP54 showed clear 'in-out' responses except for the white disc. GP58 gave continuous responses to movement of targets through its receptive field. Note that the black disc produced stronger responses than the white one. However, in the dark the white disc (surround hatched) also gave stronger responses. Downward shift of lower traces shows stimulation. N, nasal, T, temporal. These letters indicate movement direction of stimuli. Unlabeled shift shows movement in the sensitive direction, T-N.

the moment when a light spot or disc pattern was moved into or out of their RF. Other units responded continuously to moving targets. About 40% of units preferred temporonasal movement (Fig. 3B). Some units produced so-called 'in-out' firing only when targets were moved in the sensitive direction. The size of RF of the lacertilian isthmic units was larger than 3°, with the largest 120°. Eighty percent of RF centers were distributed in the contralateral lower visual field. Two binocular units were found from NI of *Gekko*. However, this has to be further examined and might be due to incomplete covering of the eyes.

We also examined tactile responses of 28 units and auditory responses of 59 units as described previously<sup>18</sup>. All of them were found to be 'pure' visual units. In 3 experiments, both visual and auditory responses were explored during advancement of an electrode to a depth of 1400 μm beneath the surface. All 14 units encountered were visual; cobalt sulphide markings indicated that 13 units were located within Imc while 1 was in the tegmentum, at some distance from the ventral border of Ipc. Thus, neither visual nor auditory units were found in Ipc. Moreover, based on correspondence of the locations of RF centers in the visual field with the recording sites marked by CoS in

Imc, it appears that the visual field is topographically mapped on Imc.

To summarize, in this study we found an indirect neuronal pathway from the tectum to Imc via NPM. NPM is located between OT and NI in the dorsolateral tegmentum. Preliminary results using anterograde transport of HRP along the optic nerve showed that NPM receives no direct retinal fibers. Previously, a tecto-pretecto-NI pathway has been found only in a teleost<sup>7</sup>. Ito et al. indicated that the nucleus pretectalis exerts inhibition on isthmic neurons. This may also take place in the reptilian tecto-NPM-Imc circuit. HRP and Golgi-Cox studies showed that Imc and Ipc are independent nuclei. The finding of photergic and scotergic units from lizards may suggest that luxotonic activity is not unique for species with unequivocal color vision<sup>8</sup>. Compared to NI of anurans<sup>16-18</sup>, those of *Gekko* and *Shinisaurus* pay greater attention to the lower visual field. This seems to be in agreement with differences in their prey-catching behavior.

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