

## Intracellular recording and morphology of tectal neurons activated by contralateral nucleus isthmi in toads

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THE nucleus isthmi (NI) in lower vertebrates and its mammalian analogue, the nucleus parabrachialis, are all visual centres. They receive their tectal input ipsilaterally and project back ipsilaterally in teleosts and birds, or bilaterally in amphibians and reptiles, as well as in mammals<sup>[1]</sup>. It is well known that NI receives its visual excitation through the ipsilateral optic tectum (OT). NI mainly exerts inhibition on the ipsilateral OT in frogs<sup>[2]</sup>. A recent study by Wang *et al.*<sup>[3]</sup> has indicated that the parvocellular (Ipc) and magnocellular (Imc) components of the pigeon NI could negatively and positively modulate ipsilaterally tectal responses, respectively. These findings have been confirmed by our microiontophoretic studies<sup>[4]</sup>.

However, nothing is known about synaptic action of the contralateral isthmotectal projection, although it is generally accepted that the amphibian NI is a relay station in the ipsilateral visuotectal projection<sup>[5-7]</sup>. The crossed isthmotectal projection has been suggested to be cholinergic<sup>[8]</sup>. Electrophysiological data concerning the functional role of acetylcholine as a transmitter or modulator within the tectum are controversial<sup>[9-11]</sup>. Therefore, this study was carried out to examine the synaptic action of the contralateral isthmotectal projection on tectal cells and their morphologies.

### 1 Materials and methods

Thirty adult toads (*Bufo bufo gargarizans*) were used in this study. The animals were immobilized with gallamine triethiodide, and the optic tecta were exposed. Anesthetic procaine was periodically applied to the wounded skin and muscle during surgical and recording sessions. Bipolar tungsten electrodes were bilaterally placed on the optic tract, and the third bipolar was inserted stereotaxically into NI (fig. 1). These electrodes were used for delivering electrical stimulation with rectangular pulses of 30–400  $\mu\text{A}$  intensity and 100  $\mu\text{s}$  duration. Micropipettes filled with 3 mol/L potassium acetate (0.5–1.0  $\mu\text{m}$  tip diameter, 20–60 M $\Omega$  impedance) were used for intracellular recordings. Postsynaptic potentials were monitored with a storage oscilloscope and stored on a tape recorder for off-line analysis.

In some experiments, micropipettes filled with 5% Lucifer yellow were used. The impaled cells were injected by passing negative current of 2–5 nA for 2–5 min to label their morphologies, and 30  $\mu\text{A}$  current was passed for 10–25 s through the stimulating electrode to verify its position within the isthmic area. After 2–7 h survival, the animal was sacrificed and

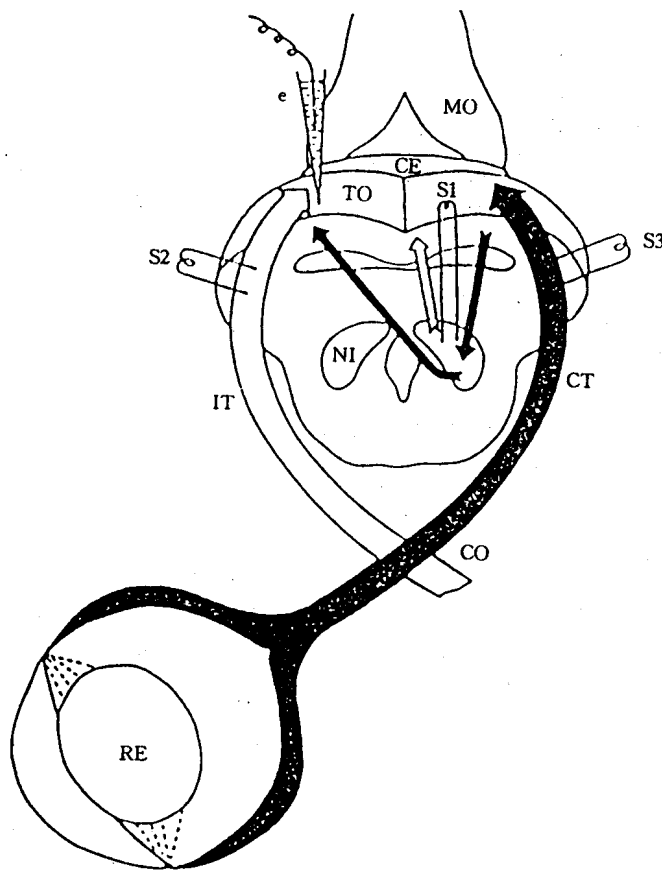


Fig. 1. Experimental set-up showing cross-section of midbrain, neuronal projections (arrows) and placement of electrodes. S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>, stimulating electrodes; e, recording pipette; CE, cerebellum; CO, optic chiasm; CT, contralateral optic tract; IT, ipsilateral tract; MO, medulla oblongata; TO, optic tectum; RE, right eye; NI, nucleus isthmi.

its brain was removed, fixed in a mixture of 3% formaldehyde and 3% glutaraldehyde, and then immersed in 30% sucrose solution overnight. Frozen sections were cut and processed for observation.

## 2 Results

Electrical stimulation of the ipsilateral optic tract evoked postsynaptic potentials in 270 tectal cells, of which 109 cells (40%) also responded to activation of the contralateral optic tract and NI. These cells had resting potentials ranging from  $-25$  to  $-60$  mV, with an average of  $-38$  mV, their recording depths ranged from 120 to 640  $\mu\text{m}$  below the tectal surface.

Following electrical stimulation of the contralateral tract, excitatory postsynaptic potentials (EPSPs) were produced in 21 of 109 cells (19%), and inhibitory postsynaptic potentials (IPSPs) in 88 cells (81%), if only considering the initial response of postsynaptic potentials. Similarly, activation of the contralateral NI evoked EPSPs in 24 cells (22%) and IPSPs in 85 cells (78%). The effect of intracellularly injected current on the recorded potentials was tested for some of the cells, which proved that these potentials were excitatory postsynaptic potentials or inhibitory postsynaptic potentials<sup>[2]</sup>. By comparing the postsynaptic responses evoked by the tract stimulation with those by the isthmic stimulation, it was found that they were often similar in terms of polarity, waveform and time course (fig. 2). All tectal cells responding

to the tract stimulation also responded to the isthmic stimulation contralaterally. None of them only responded to the contralateral tract stimulation. It implies that activation of the optic tectum was delivered to the contralateral tectum through the tectoisthmotectal pathway. The average latencies of EPSPs were 26.3 ms for the tract stimulation and 14.1 ms for the isthmic stimulation, those of IPSPs were 32.2 and 19.6 ms respectively. Therefore, the average latency for the tract stimulation was about 12 ms longer than that for the isthmic stimulation. It is obvious that this time difference is taken mainly by activation traveling from the tract stimulation site to NI. A comparable time is taken by the contralateral isthmotectal pathway, suggesting that at least EPSPs in tectal cells are monosynaptically evoked.

Of 25 tectal cells labelled with intracellular Lucifer yellow, 16 (64%) were localized in layer 6 and others in layers 4, 5, 7 and 8 (fig. 3). 17 cells were identified as pear-shaped cells and classified into two groups: one group of cells had a narrow dendritic field (for example, fig. 3b). The cells with a few dendritic branches might be incompletely filled for some reasons; another group had a broad field (for example, fig. 3h). Other 8 cells were ganglionic (fig. 3 a, g, n, r, s, w, x and y). They gave more than two primary dendrites, which could form a broad field of up to 800  $\mu\text{m}$  across, with some dendrites spreading into the contralateral tectum. The axons of some ganglionic cells were visible and could be traced for a long distance. Seven of 25 injections (28%) resulted in multilabelings, each consisting of up to 7 cells. Generally speaking, the primary cell in a multilabeled group had stronger fluorescing intensity than its secondary cells, which appeared to be incompletely filled with the dye. Cells of a multilabeled group could form a column-like arrangement (for example, figure 3t).

It was difficult to correlate the electrophysiological properties of the recorded cells with their morphological features stained with Lucifer yellow, based on a small number of cells. For example, cells c and s in fig. 3 were very different in their laminar locations and morphological features; they produced, however, a sequence of EPSP-IPSP to the tract stimulation and the isthmic stimulation. On the other hand, cell y always produced a single IPSP response to both stimulations, and its location and morphology were similar to those of cell s. Irrespective of this, cellular labellings also can provide some evidence for intracellular impalement of cells.

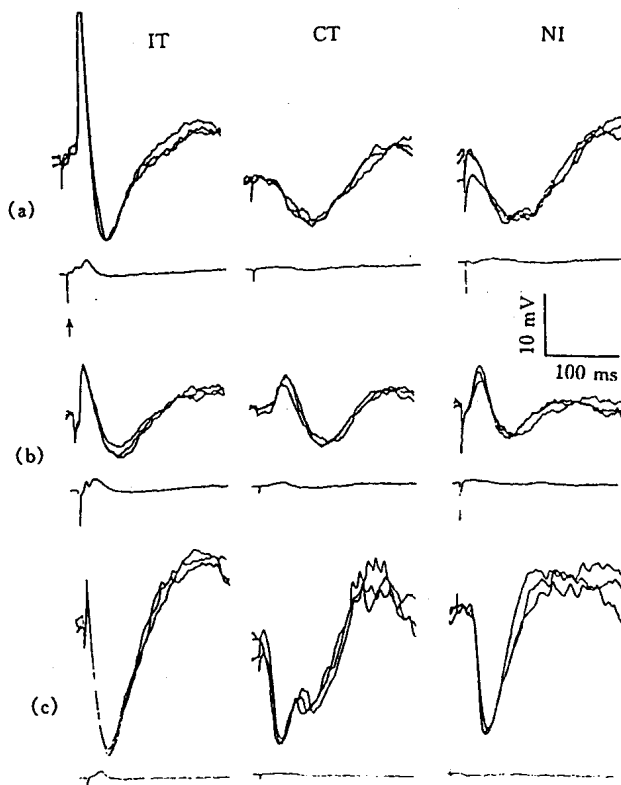


Fig. 2. Postsynaptic responses of three tectal cells ((a)—(c)) to electrical stimulation applied to the ipsilateral optic tract (IT), contralateral tract (CT), and to nucleus isthmi (NI). Second traces are field potentials recorded just outside of the recorded cells. Arrow points to artifact elicited by electrical stimulation. Scales: 10 mV, 100 ms.

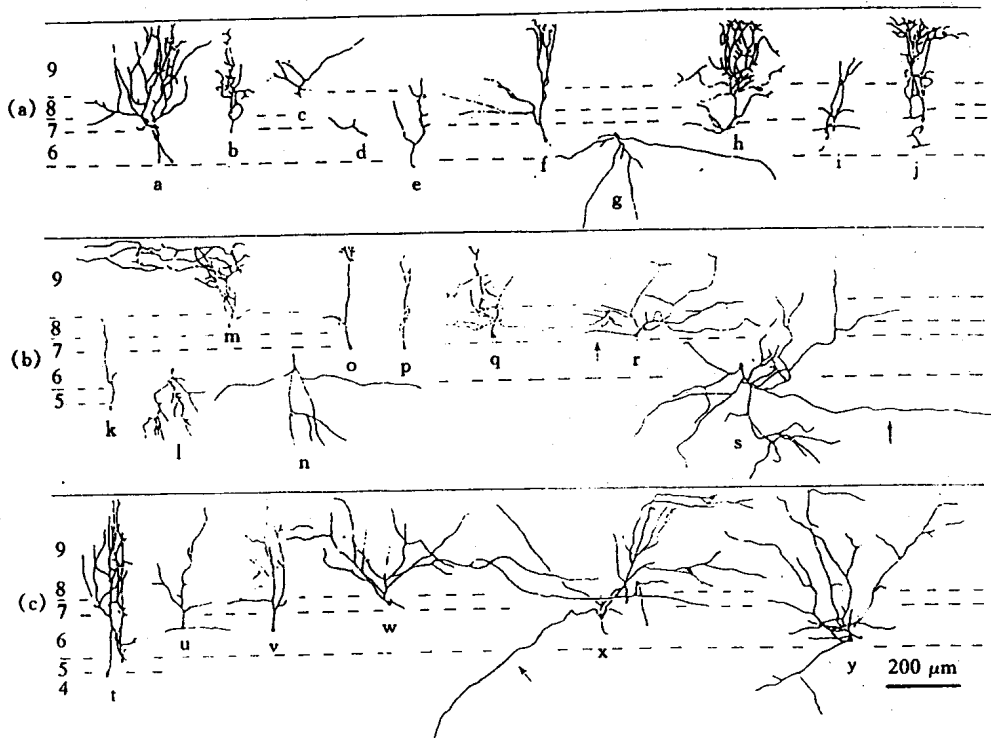


Fig. 3. Morphologies and laminar distribution of 25 tectal cells labelled with Lucifer yellow after being intracellularly recorded. Note that cases a, i, j, m, t, x and y show multilabeled cells in a column-like structure. The figure consists of three continued parts (A, B, C). Numerals at the left margin represent tectal laminations. Arrows point to axons. Scale bar; 200  $\mu\text{m}$ .

### 3 Discussion

To our knowledge, this report is the first to study the functional significance of the crossed isthmo-tectal projection by using intracellular recording and staining techniques. The present results show that electrical stimulation of the contralateral NI elicits EPSPs in 22% and IPSPs in 78% of tectal cells recorded. Similar results have been obtained in tectal cells responding to electrical stimulation of the ipsilateral NI<sup>[2]</sup>. These findings about bilateral inhibition on tectal cells are supported by the fact that removing the isthmoc input by electrolytic lesions results in global disinhibition in both tecta<sup>[5]</sup>.

Several extracellular studies have indicated that tectal binocular cells receive their ipsilateral information relayed by the contralateral NI<sup>[5, 6, 12]</sup>. This study shows that about 20% of isthmoc cells relay excitatory information from the contralateral tectum to the ipsilateral tectum. This crossed isthmo-tectal pathway may be cholinergic<sup>[8, 13]</sup>. Ionophoretically applied acetylcholine (ACh) could excite most of cells in the superficial tectum<sup>[11]</sup>. However, this is in disagreement with behavioral tests, indicating that there may be an inhibitory cholinergic system in the amphibian tectum, which plays an important role in visually guided behavior<sup>[9, 10]</sup>.

It appears that there exists only one neural pathway transmitting information from one tectum to another. It is mediated by NI through the postoptic commissure<sup>[5, 12, 14]</sup>. Although some studies have suggested that there exists direct intertectal projection<sup>[15]</sup>, but it has not been confirmed by recent tracing studies<sup>[16, 17]</sup>. However, Lazar *et al.*<sup>[18]</sup> reported that the

dendrites of some tectal neurons might extend across to the contralateral tectum. This finding is confirmed by our intracellular stainings of a tectal cell, which could receive its input directly from the contralateral tectum. The present study does not show the functional significance of this connection.

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