

# Postsynaptic potentials and morphology of tectal cells responding to electrical stimulation of the bullfrog nucleus isthmi

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## Abstract

Postsynaptic responses of tectal cells in the bullfrog (*Rana catesbeiana*) were intracellularly recorded following electrical stimulation of the optic tract and the nucleus isthmi, and fluorescent dye, Lucifer yellow, was injected into some of the impaled cells to show their morphologies. Two main response types were found: The first type was an EPSP followed by an IPSP, and the second type was single IPSP. The first type predominates in cells responding to the optic tract stimulation and the second type prevails in cells responding to the isthmic stimulation. Fifteen cells stained with Lucifer yellow were localized in layer 6 (11 cells), layer 7 (1 cell), and layer 8 (3 cells). They were mainly identified as pear-shaped cells, large ganglionic cells, and stellate cells. Three injections demonstrated “dye-coupling,” which labeled up to six cells following one injection. Comparisons of postsynaptic potentials with cellular morphologies suggested that the nucleus isthmi could directly excite large ganglionic neurons in layer 6. Synaptic mechanisms for strong isthmic inhibition on the tectal neurons remain unknown.

**Keywords:** Electrical stimulation, Intracellular staining, Postsynaptic potential, Tectal neuron, Nucleus isthmi, Bullfrog

## Introduction

The neuroanatomy and electrophysiology of the nucleus isthmi (NI) in lower vertebrates and of its mammalian analogue, the nucleus parabigeminalis, have been extensively studied (see reviews: Gruberg, 1983; Wang, 1988). This nucleus receives its input from the ipsilateral tectum and projects back ipsilaterally in teleosts (Ito et al., 1981, 1982; Sas & Maler, 1986, found bilateral projection in a fish) and birds (Hunt & Kunzle, 1976; Hunt et al., 1977; Kunzle & Schnyder, 1984), or bilaterally in amphibians (Grobstein et al., 1978; Gruberg & Udin, 1978; Gruberg & Lettvin, 1980; Wang et al., 1983b) and in reptiles (Wang et al., 1983a; Kunzle & Schnyder, 1984), as well as in mammals (Graybiel, 1978; Baleydiere & Magnin, 1979; Sherk, 1979b; Watanabe & Kawana, 1979; Mendez-Otero et al., 1980; Linden & Perry, 1983; Rolden et al., 1983; Jen et al., 1984; Kunzle & Schnyder, 1984). It also has neural connections with the nucleus pretectalis (Ito et al., 1981, 1982) and the torus semicircularis (Sas & Maler, 1986) in teleosts, and with the an-

terodorsal tegmental nucleus in amphibians (Udin, 1987), with the nucleus profundus mesencephali in reptiles (Wang et al., 1983a), as well as with the dorsal lateral geniculate nucleus in mammals (Graybiel, 1978; Harting et al., 1986; Hashikawa et al., 1986).

Electrical stimulation applied to the optic tectum of the optic nerve could elicit field and unitary potentials from the NI in teleosts (Williams & Vanegas, 1982; Williams et al., 1983) and in amphibians (Vinogradova & Manteifel, 1977). Electrophysiological studies on several species of vertebrates have shown that NI and its mammalian analogue are all visual centers that receive visual information from the tectum (Sherk, 1978, 1979a, b; Vinogradova & Manteifel, 1979; Wang et al., 1981, 1982, 1983; Yan & Wang, 1986). The tectoisthmic pathway may be cholinergic (Hung et al., 1976; Felix et al., 1985; Wang et al., 1985; Li et al., 1987). There is some evidence suggesting that the NI may also receive an inhibitory input from the nucleus pretectalis in the fish (Ito et al., 1982), or from the nucleus profundus mesencephali in the lizard (Wang et al., 1983a).

However, until now very little is known about the nature of the projection from the NI to the tectum. Several neuroanatomical studies have indicated that the isthmotectal fibers terminate in register with the retinotectal terminations (Hunt et al., 1977;

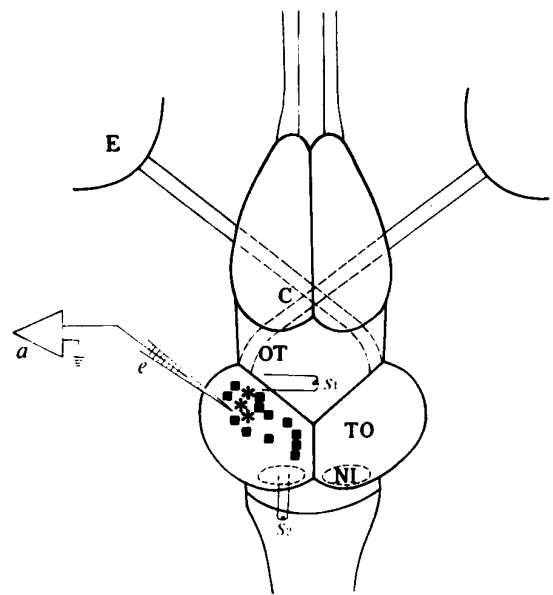
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Graybiel, 1978; Gruberg & Udin, 1978; Gruberg & Lettvin, 1980; Kunzle & Schnyder, 1984). Furthermore, this pathway has been suggested to be cholinergic (Ricciuti & Gruberg, 1985; Fite & Wang, 1986; Mufson et al., 1986; Wang et al., 1986a,b; Li et al., 1987). However, electrophysiological data pertaining to the functional role of acetylcholine as a transmitter within the tectum are controversial (Stevens, 1973; Hock, 1983; Fite & Wang, 1986; Wang et al., 1986b). Behavioral tests obtained on amphibians whose bilateral NI were lesioned also are in disagreement (Collett & Udin, 1983; Caine & Gruberg, 1985). Electrolytic lesions of the NI resulted in disinhibition of tectal cells (Glasser & Ingle, 1978). Therefore, it is necessary to make intracellular recordings from tectal cells that receive their input from the NI. Even though the cell bodies of tectal neurons are very small in size, Matsumoto and his collaborators (Matsumoto & Bando, 1980; Antal et al., 1986; Matsumoto et al., 1986; Nagano et al., 1988; Matsumoto, 1989) have successfully recorded intracellular responses of tectal cells in amphibians to electrical stimulation of the optic nerve or the optic tract, as well as to visual stimulation, and stained some of the electrophysiologically identified tectal neurons. To further investigate neuronal circuitry in the amphibian midbrain in general and reveal the nature of the isthmotectal projection in particular, we made intracellular recordings from tectal cells in frogs following electrical stimulation of the optic tract and of the nucleus isthmi and stained some of the impaled neurons. The relationship between the electrophysiological properties and the cellular morphologies of the tectal neurons was analyzed.

## Methods

The experiments were performed on 22 adult bullfrogs (*Rana catesbeiana*) having 100–250 g body weight (b.w.). Conventional procedures for exposing the optic tectum were carried out. Briefly, the frog was anesthetized with ether and paralyzed by an injection of succinylcholine (5 mg/100 g b.w.) in the dorsal lymphatic sac, and then placed on a metal frame. The body was covered with a wet gauze. The skull was opened by using a dental burr-drill and the dura overlying the tectum removed. The pressure points and head wounds were infiltrated with a local anesthetic. A tungsten bipolar electrode was placed on the dorsal optic tract (OT), and a micropipette was stereotaxically inserted into the isthmic area to record field and unitary potentials evoked by electrical stimulation of the OT for determining the exact location of the NI. The stereotaxic coordinates used for penetrating the NI were as follows, *X* (mediolateral) = 900–1100  $\mu\text{m}$ , *Y* (caudorostral) = 400–600  $\mu\text{m}$ , and *Z* (dorsoventral) = 1800–2200  $\mu\text{m}$ , with a zero point being at the apex of the triangle between the cerebellum and the optic tecta, where the two tecta meet together. The micropipette was then replaced by a second tungsten bipolar electrode. Experimental arrangement is shown in Fig. 1.

For electrical stimulation of the OT or the NI, rectangular pulses of 100–400  $\mu\text{A}$  in intensity and 100  $\mu\text{s}$  in duration were delivered. Electrodes for intracellular recording were micropipettes filled with 2 M potassium citrate. The tip of the electrodes was broken by touching it against the edge of a glass slide with a micromanipulator under a microscope. The tip diameter was about 0.5–1  $\mu\text{m}$  and the impedance 20–50 M $\Omega$  after tip breaking. Tectal neurons were impaled by applying brief positive current pulses of about 20 nA. Intracellular impalement



**Fig. 1.** Schematic experimental arrangement and recording positions (squares and asterisks) of 15 neurons stained with Lucifer yellow on the dorsal surface of the left tectum. Asterisks in caudorostral extent signify the recording positions of three large ganglionic cells *m*, *n*, and *o* in Fig. 5. C: chiasm; E: eye; NI: nucleus isthmi; OT: optic tract; TO: tectum opticum; a: preamplifier; e: electrode; and S1, S2: stimulating electrodes.

was signalled by a sudden d.c. drop of 30–70 mV, and by the appearance of postsynaptic potentials or occasionally action potentials. Intracellular responses evoked by electrical stimulation applied to the NI and the OT were fed into a preamplifier (MEZ-8101, Nihonkoden), whose output was monitored on the screen of a digital storage oscilloscope (VC-10, Nihonkoden) and stored on the magnetic tape of a data recorder (DFR-3515, Sony). The responses were played back onto the oscilloscope and an X–Y recorder (WTR-751, Watanabe) for their display and superposition.

After intracellular recordings were made, 30- $\mu\text{A}$  current for 10–25 s was passed through the stimulating electrode in order to verify its position. In some cases, the tip position of the reference pole (positive pole) of the electrode was also marked by passing smaller current. In the second part of the experiments, micropipettes filled with 5% Lucifer yellow (CH, dilithium salt, Sigma, St. Louis, MO) were used. Following intracellular recordings, the tectal neurons were injected by passing negative current of 2–5 nA through the electrodes for 2–5 min. After a 4-h survival, the brain was removed and fixed in a mixture of 3% formaldehyde and 3% glutaraldehyde, and then immersed in 30% sucrose in phosphate buffer solution overnight. Frozen sections were cut at 80  $\mu\text{m}$ , mounted, and dehydrated. Serial sections containing the stained neurons were observed and photographed with a fluorescence microscope (Olympus, B filter). The neurons were graphically reconstructed based on the photomicrographs taken from the sections at different depths. The relationship between the intracellular responses and the cellular morphologies of the stained neurons was analyzed.

## Results

### Intracellular responses

Electrophysiological responses of 82 tectal neurons to electrical stimulation of the OT and the NI were intracellularly recorded from 22 frogs, and 15 neurons of these were intracellularly marked with Lucifer yellow to show their tectal locations and cellular morphologies. The membrane potentials of the impaled neurons ranged between  $-30$  mV and  $-70$  mV ( $-44 \pm 13$  mV; mean  $\pm$  s.d.). After impaling the neurons, we waited at least a few minutes so that the neuron recovers from the damage of the membrane. Recording of intracellular responses needed an additional 2–3 min. During this period, the resting potential was stable, and those neurons which showed considerable changes in resting potential were eliminated from the present sample. After the staining, however, some neurons had greatly deteriorated or lost the resting potential, but the staining was successful even in such cases. In five frogs, 12 cells (32%) were found responding only to the OT stimulation, and these cells were omitted from the analysis. Electrolytic lesions indicated that the electrode tip (negative pole) used for electrically stimulating the NI was all localized within the nucleus in 22 frogs (Fig. 2). The tip of the reference electrodes was adjacent to or within the NI. It appeared that the electrical stimulation was, at least in great part, restricted to the nucleus.

Some different combinations of depolarizing and hyperpolarizing potentials could be observed following both stimulations. Those potentials were graded in amplitude and effects of

intracellularly applied current were tested for some of the recorded cells, which proved that they were excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs), respectively (see Matsumoto & Bando, 1980). Examples of responses of tectal neurons to both the OT and the NI stimulations are shown in Fig. 3.

Responses to the OT stimulation were similar to those in the former experiments (Matsumoto & Bando, 1980; Matsumoto et al., 1986). If we consider only the first two components (EPSP/rebound excitation: E, IPSP: I), the OT stimulation elicited EI-type response (Fig. 3C) in 46 cells (56%), I-type response (Fig. 3D) in 15 cells (19%), IE-type response (Fig. 3B) in 10 cells (12%), II-type response in 10 cells (12%), and E-type response in 1 cell (1%). In most EI-type responses, the initial EPSPs were small in amplitude and were overcome by the following IPSP with long duration and large amplitude, which made the depolarizing phase very short and kept the membrane potential under threshold. The initial IPSPs in I- or IE-type response had also long duration and large amplitude.

The NI stimulation evoked I-type response (Fig. 3C and 3D) in 47 cells (57%), EI-type response (Fig. 3A and 3B) in 24 cells (30%), II-type responses in 8 cells (10%), IE-type response in 2 cells (2%), and E-type response in 1 cell (1%). Responses to the NI stimulation also showed that initial EPSP was interrupted by the following large IPSP.

In comparison of OT-induced responses with NI-induced responses, considering only the initial potentials, 46 cells (56%) produced polarity-identical responses (Fig. 3A and 3D), while

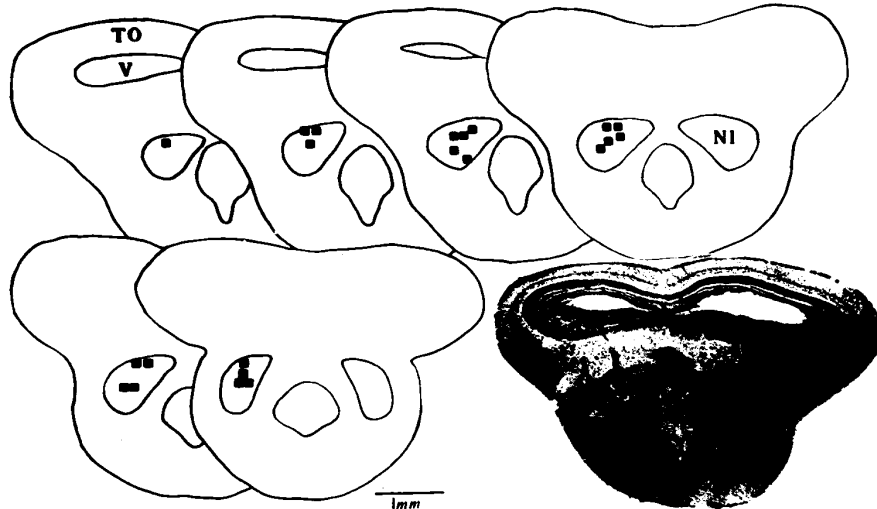
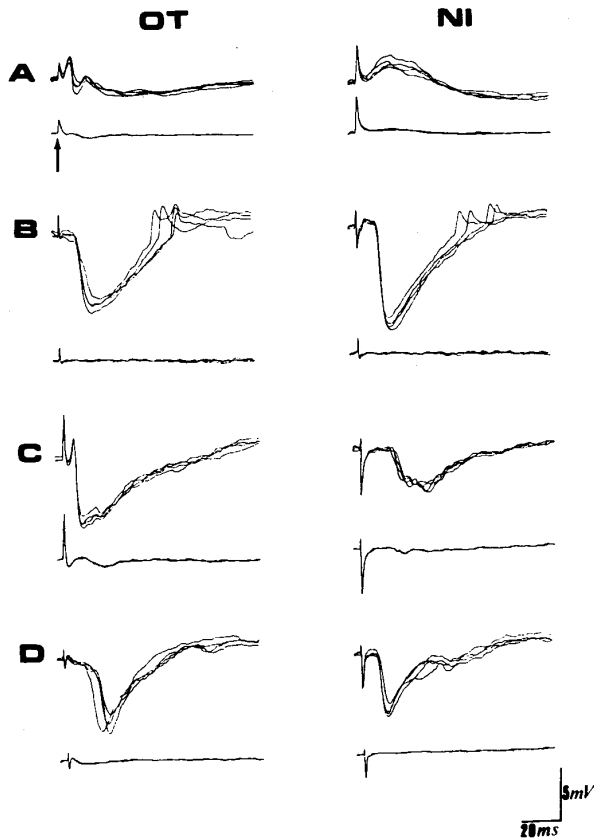


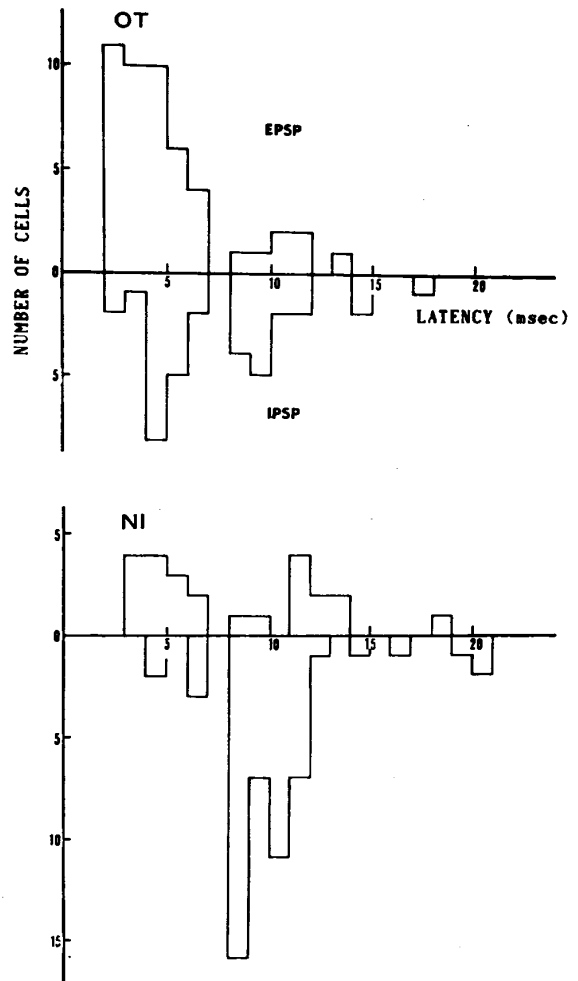
Fig. 2. Localization of stimulating electrode tips marked by electrolytic lesions (squares) within the nucleus isthmi (NI) in 22 frogs. Two lesions are shown by asterisk (active pole) and arrow (reference pole) in a photomicrograph of a transverse brain section at the NI level. TO: tectum opticum; and V: ventricle.



**Fig. 3.** Intracellular recordings from four tectal neurons (A-D) responding to electrical stimulation of the optic tract (OT) and the nucleus isthmi (NI). A: Responses with initial excitation for both stimulations (EIEI type for OT and EI type for NI). B: The OT stimulation elicited an IPSP followed by rebound excitation, while the NI stimulation produced a very small EPSP followed by a large IPSP and then rebound excitation. C: A neuron showing an EPSP (I type) to the OT stimulation and a pure IPSP (I type) to the NI stimulation. D: A neuron responding with an IPSP to both stimulations (I type). Lower traces under each postsynaptic potential show field potentials recorded just outside of the impaled neuron. The onset of the electrical stimulation is indicated by arrow in A.

36 cells (44%) had polarity-inverted ones (Fig. 3B and 3C). There were five cells that responded with similar postsynaptic potentials to the two stimulations.

Latencies of the initial potential were measured for both the OT and the NI stimulations. The result is shown in the histograms in Fig. 4. In the OT stimulation, the histograms for either EPSP and IPSP have a prominent group between 2 and 7 ms. Judging from the former findings (Matsumoto & Bando, 1980; Nagano et al., 1988), OT-induced EPSPs with shorter latencies of this group should be monosynaptic. A few cells were found to have very short IPSP latencies (2-3 ms) to the OT stimulation, presumably receiving a direct inhibitory input from the contralateral retina. The rest of the IPSPs in the group are considered to be disynaptic or polysynaptic. Histograms for the NI stimulation show a different distribution of latencies. Considering the longer distances between the NI deep in the caudal mid-

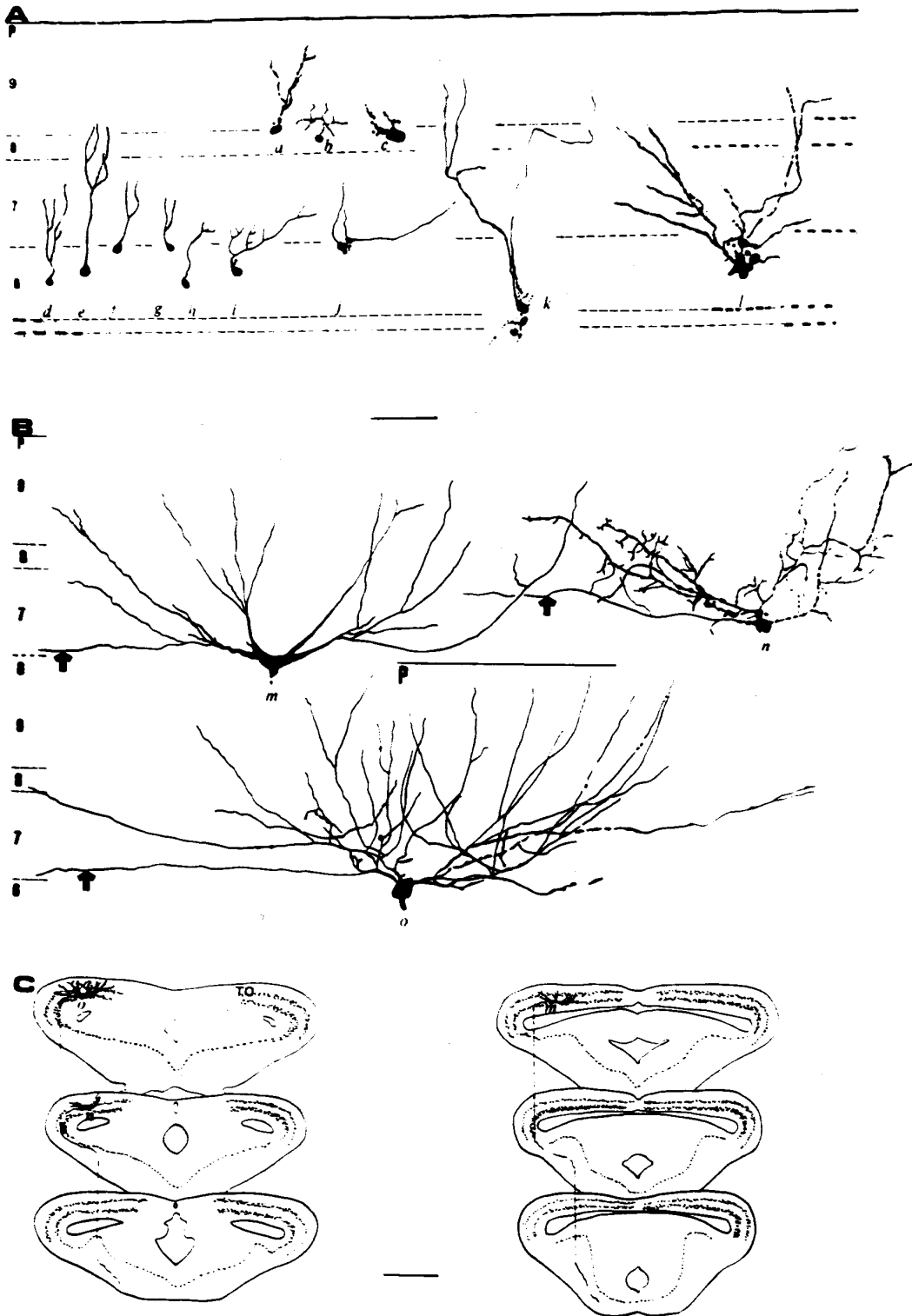


**Fig. 4.** Latency histograms of postsynaptic potentials in tectal cells responding to electrical stimulation of the optic tract (OT) and the nucleus isthmi (NI). Upward histograms show EPSP latencies and downward histograms IPSP latencies. In the histogram for the NI stimulation, six cells with longer latencies (23-50 ms) are omitted.

brain and the recording sites at the rostral part of the tectum, EPSPs with the shortest latencies in the histograms may be considered to be monosynaptic. There is a prominent peak in the IPSP histograms between 8 ms and 13 ms, but there are a very few shorter latencies. Since the duration and the amplitude of NI-induced IPSPs are much bigger than those of EPSPs in many of the recorded cells, it was postulated that the NI gives a strong inhibitory effect on the tectum.

#### Cellular morphologies

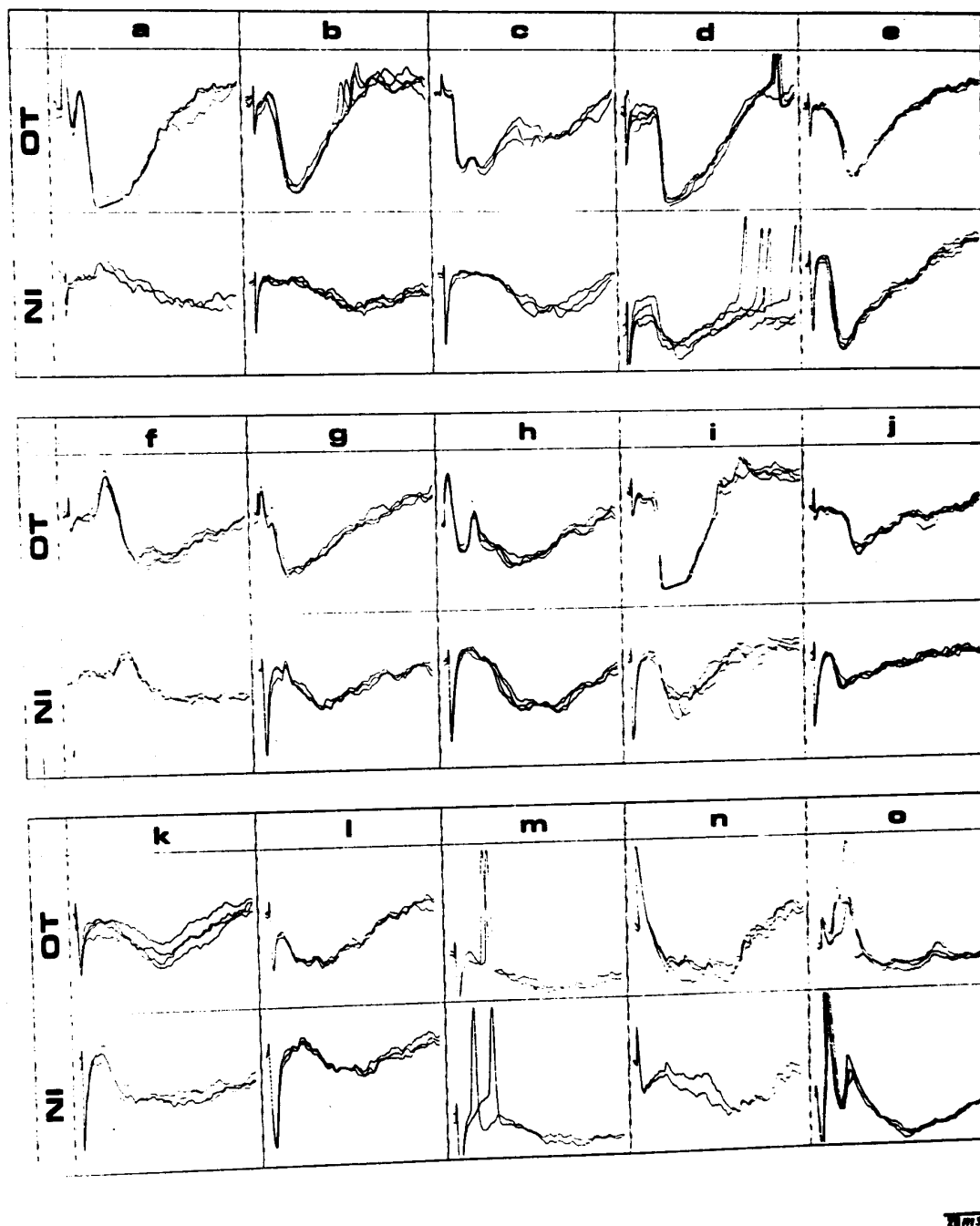
Twenty-four tectal cells were intracellularly injected with Lucifer yellow after being recorded. Fifteen injections were successful and the locations of those labeled cells are shown in Fig. 1, and the morphologies in Fig. 5. Three injections produced multiple labelings each consisting of 2-6 cells in a cluster (Fig. 5). The largest distance between the primary cell and



**Fig. 5.** Cellular morphologies and laminar distribution of tectal cells intracellularly stained with Lucifer yellow (A,B). Note that cases *j*, *k*, and *l* are multiple cell labelings. Cells *m*, *n*, and *o* are large ganglionic cells, whose axons (arrows) could be traced for a considerable distance. Their localization and axons' traveling course are shown in transverse brain sections (C). Numerals at the left margin and letter P indicate the tectal laminations and the pia matter. Alphabetical letters mark the stained cells, whose corresponding postsynaptic responses are shown in Fig. 6. Scale bars in A,B = 100  $\mu$ m; C = 1 mm.

the secondary cells was  $30\ \mu\text{m}$ . As shown in Fig. 5, three multilabeled cells were located in single (*l*), two (*j*), or three (*k*) tectal layers. In case *k*, three marked cells were distributed in layers 4, 5, and 6. The cell bodies in the same group were al-

most identical in fluorescing intensity. On the other hand, the process-free space between the cell bodies was devoid of the fluorescent dye. However, the primary cell and its secondaries could be differentiated based on the filling of their processes in



**Fig. 6.** Postsynaptic potentials intracellularly recorded from tectal neurons that were stained with Lucifer yellow after being recorded. The field potentials are not shown, but they did not make significant contribution to the intracellular responses (see Fig. 3 for comparison). Alphabetic letters signify the neurons corresponding to those sketched in Fig. 5. NI: isthmus stimulation; and OT: optic tract stimulation.

some cases. A typical example is given in Fig. 5*k*, showing that the primary cell had a long, branched apical dendrite and its secondaries had dendrites that were not completely filled. Occasionally, some connecting structure could be observed between the cells within the same group (Fig. 5*j, l*).

Of 15 cells marked with Lucifer yellow, 11 cells were located in layer 6, 1 in layer 7, and 3 in layer 8. Most of cells localized in layer 6 were pear-shaped cells (Fig. 5*d-j, k*), which originated an apical dendrite going upward to the superficial layers. Their dendritic branches terminated within layer 7 or layer 9. Cells *j* and *l* stained together with their secondary cells might be identified as ganglionic cells. In both cases, there seemed to be somatodendritic or somatosomatic connections between the cells. Two large ganglionic cells were stained in the superficial portion of layer 6, at the border between layer 6 and layer 7 (Fig. 5*m, o*). They had quite large multipolar somata measuring 40–60  $\mu\text{m}$  in their long dimension and broad dendritic field extending 900–1300  $\mu\text{m}$  in the mediolateral direction. The cell bodies gave off 3–4 primary dendrites, which arborized further as running horizontally, obliquely, and upwards. Their dendrites were mainly terminated in layer 9 and some of them just ended below the pia surface. An axon was seen to emerge in layer 7 from one of the primary dendrites. These axons run laterally within layer 7 and then turn to the ventrolateral direction (Fig. 5*C*). They could be followed for a long distance in the mesencephalic tegmentum, and presumably joined in the tectobulbosplinal tract. Another large ganglionic cell (Fig. 5*B, n*) was stained in the deeper portion of layer 7. Its soma sent out a few primary dendrites, from which originated many tortuous branches. Some branches could be observed close to the pia surface. These dendrites bore numerous varicosities. An axon had its origin from the cell body; it branches several times and runs obliquely to the upper portion of layer 7. Three cells were localized within ill-defined layer 8. In Fig. 5, cell *a* appeared to be a pear-shaped cell, but it had thicker dendrites bearing varicosities, all of which terminated in layer 9. Cells *b* and *c* were stellate neurons. Cell *b* had relatively small soma and gave rise to a single process repeatedly branching within layers 8 and 9. Cell *c* had large soma (30  $\mu\text{m}$ ) and few thicker, shorter dendrites bearing varicosities. It is clearly seen from a photomicrograph that the somatic center of cell *c* has much stronger fluorescing intensity than the other parts of the cell, suggesting that the center might be the impalement site of an electrode.

#### *Relationship between responses and morphologies*

It is difficult to establish a correlation between electrophysiological properties (Fig. 6) and morphological features (Fig. 5) of the recorded cells based on a relatively small number of cells. However, some information emerges from this study. As mentioned above, neurons *m* and *o* had several morphological features in common. Both of them responded to the OT stimulation and to the NI stimulation with an EPSP–IPSP (EI) sequence. Their initial EPSPs usually could produce a spike following electrical stimulation. Their responsive latencies to the OT stimulation (11.3 and 4.5 ms, respectively) were longer than those to the NI stimulation (3.2 and 4.0 ms, respectively). Neuron *n* was distinguished from neurons *m* and *o* in their morphological features, and it responded to both stimulations each with only IPSP (I type). The IPSP elicited by the OT stimulation had much shorter latency than that by the NI stimulation.

Neurons *j, k*, and *l* were the primary cells of three multila-

beled groups of cells. The first two responded to both electrical stimulations with a single IPSP, whose latency to the OT stimulation (14.8 and 11.4 ms, respectively) was longer than that to the NI stimulation (6.8 and 9.1 ms, respectively). Neuron *l* did not follow this fashion. It responded to the OT stimulation with an IPSP (I type) and to the stimulation with an EPSP–IPSP sequence (EI type). The former response had much shorter latency (4.5 ms) than the latter one (12.6 ms).

The pear-shaped cells whose somata were in layer 6 could be divided into two groups by considering the first component of postsynaptic potentials: an inhibitory and an excitatory group. Neurons *e* and *i* in the first group responded to the OT stimulation with two successive IPSPs (II type), of which the initial one was much smaller than the late one in amplitude, and to the NI stimulation with a single large IPSP (I type). OT-induced responses had shorter latency (2.8 and 3.4 ms) than NI-induced responses (8.5 and 11.4 ms). In the excitatory group, neurons *f* and *g* responded to both stimulations with almost identical EPSP–IPSP sequence (EI type). Also, shorter latency occurred with the OT stimulation (4.5 and 12 ms vs. 8.5 and 18 ms). On the contrary, neuron *d* produced an identical IE-type response (note action potentials at rebound phase) following the two stimulations.

It is interesting to note that we did not find large ganglionic cells in layer 7 (other than the border area between layers 7 and 6) and layer 8. In the former experiment, large ganglionic cells in these two layers constitute the majority of the sampled large ganglionic cells (Antal et al., 1986). This finding suggests that those large ganglionic cells are not involved in isthmotectal information processing. It is also suggested from the aforementioned comparison that the NI monosynaptically excites large ganglionic neurons in layer 6. However, the inhibitory pathway could not be figured out from the present analysis. Another conclusion is that many tectal neurons seem to have common circuits for the responses elicited by the OT and the NI stimulations.

#### **Discussion**

This is the first intracellular study on the functional significance of the isthmotectal pathway in amphibians. It is found that about 70% of intracellularly impaled cells in the rostral half of the optic tectum respond to both the OT and NI stimulations. It might be one of the reasons why classification percentage of responsive type of the tectal cells is somewhat different from that obtained following only the OT stimulation (Matsumoto & Bando, 1980). For example, very few large ganglionic cells could be found in the present study. There are also some differences in classification percentage between the present study and the previous works on pigeons (Hardy et al., 1985; Leresche et al., 1986) due to different ways of stimulation, samples of neurons, and species of animals used. Nearly 60% of tectal cells recorded receive their excitatory input (initial response) from the retinal ganglion cells, followed by an inhibitory input that may originate from GABAergic interneurons (Roberts & Yates, 1976; Freeman & Norden, 1984) possibly by means of the GABA receptor-mediated synapses (Soltesz et al., 1989). There might be other possibilities, for example, that this inhibition has its origin from the pretectal nuclei because these nuclei are activated by electrical stimulation of the optic nerve (Matsumoto, 1989). The other cells mainly receive inhibitory input, most of which appear to be disynaptic or polysynaptic to the retina because of

their longer latencies. However, two tectal cells have short latencies (2.2 and 2.5 ms) suggesting that they may receive direct inhibitory input from the retinal ganglion cells (Hardy et al., 1985; Leresche et al., 1986).

One of the main results of this study is that stimulation of NI exerts inhibition on nearly 70% of and excitation on about 30% of the recorded cells by considering the initial response. Following the NI stimulation, IPSPs have long latencies and duration, while EPSPs are interrupted by an IPSP shortly after their onset. These IPSPs could be used to explain the results obtained by electrolytic lesions of the nucleus that the removal of the isthmotectal input results in global disinhibition of the tectum (Glasser & Ingle, 1978). Selective-uptake studies on pigeons (Hunt & Kunzle, 1976; Reubi & Cuenod, 1976; Hunt et al., 1976, 1977) lend some support to this notion. Several studies have suggested that the isthmotectal pathway in amphibians (Ricciuti & Gruberg, 1985; Wang et al., 1985; Li et al., 1987) and the parabigeminothalamic pathway in mammals (Mufson et al., 1986) are cholinergic. Although tectal cells responsible for orienting, jumping, and snapping behaviors are inhibited by acetylcholine (ACh) (Stevens, 1973; Hock, 1983), however, these results could be accounted for on the basis of very high concentrations of cholinergic substance used in both studies (Freeman & Norden, 1984). On the other hand, microiontophoretically applied ACh can play its excitatory role within the tectum in frogs (Fite & Wang, 1986) and in pigeons (Wang et al., 1986b). Therefore, it is hypothesized that ACh mediates EPSPs elicited in tectal cells by the NI stimulation.

The optic tectum is the main visual center of the frog, which receives input directly from the retina. The optic nerve is composed of four different classes of physiological types with different conduction velocities. Visual information of classes 1 and 2 is carried by unmyelinated fibers, and classes 3 and 4 by myelinated fibers (Lettvin et al., 1959). Histograms in Fig. 3 show that there are two main groups in the latencies of the first response for both OT and NI stimulation (less than 7 ms and 8–15 ms). There are two possibilities to explain the existence of two separate groups in latencies: responses with longer latencies are produced by tectal intrinsic circuit, or, alternatively, two groups of afferent fibers with different conduction velocities are involved. Although we did not measure the conduction velocities in the present study, judging from the former experiment (Matsumoto & Bando, 1980), the first group of OT stimulation seems to fit the responses elicited by myelinated fibers, and the second group to the responses by unmyelinated fibers. Distribution of caliber diameter (1–3  $\mu\text{m}$ ) of isthmotectal fibers has been reported (Gruberg & Udin, 1978), and it may also be possible to attribute the separation of latencies for NI stimulation to difference in conduction velocities. An alternative possibility is that the responses in the second group are produced by the output of the first group through neural chains. In this case, however, the number of synapses involved should be two or more considering the difference in the mean latencies of both groups. If we accept this possibility, separation of the latencies should have been much less prominent or rather continuous, since we could have recorded any cells in the neural chain. Anatomical studies have shown that the ipsilateral isthmotectal fibers are distributed in the superficial layers of the tectum, consistent with the retinotectal terminals (Gruberg & Udin, 1978; Gruberg & Lettvin, 1980). This finding and the feature of the latency histograms suggest that retinotectal and isthmotectal systems use some common circuit, but the latter more effec-

tively activates inhibitory units. Since we did not measure the latencies of the second response, further analysis for detailed neural connections in the optic tectum could not be performed, and it remains to be studied in the future.

The second main result of the study is the demonstration of morphologies of tectal cells that receive their input from both the retinal ganglion cells and the NI. Lucifer yellow is a satisfactory intracellular dye, with which a higher percentage of injected cells (about 70%) can be successfully stained and their somata, dendrites, and occasionally axons can be clearly demonstrated (in some small cells, filling is insufficient). The morphological classification of the stained cells is similar to the tectal cell types shown by Golgi impregnation (Potter, 1969; Székely & Lázár, 1976). Most of the stained cells are located in layer 6. On the other hand, numerous cells of this layer project their axons to the NI (Gruberg & Lettvin, 1980; Wang et al., 1983b). Therefore, there may be some circuits or loops established between these receiving and projecting cells in layer 6. If so, a component evoked polysynaptically by the nucleus would probably appear on the postsynaptic potential recorded following the OT stimulation. However, any such component is difficult to distinguish it from others in this study. The pear-shaped cells in layer 6 might also project rostrally to the diencephalon or the thalamus, or caudally to the medulla oblongata or the spinal cord (Lázár et al., 1983). The morphological features of cell *a* in Fig. 5 show that it might be a rostrally projecting neuron to the diencephalon (Lázár et al., 1983). The other two stellate cells in layer 8 have small dendritic fields and could be regarded as local circuit neurons. It is interesting to note that the two large ganglionic cells located at the superficial portion of layer 6 send out their long axons joining in the caudal efferent pathways of the tectum. These axons may travel to the contralateral side to participate in the tectobulbospinal tract (Weerasuriya & Ewert, 1981; Lázár et al., 1983). This is supported by the largest antidromic field potential recorded at the border between layers 6 and 7 of the tectum when electrical stimulation is applied to the contralateral caudal medulla (Satou & Ewert, 1985). The axon of the large ganglionic cell stained in layer 7 gives off two axon collaterals representing its connection with 2–3 regions. Its main axon might join in either the rostrally efferent pathways or the caudally efferent pathways of the tectum. However, it projects more probably to the ipsilateral side (Lázár et al., 1983). This cell responds with IPSPs to electrical stimulation of the OT and the NI, appearing to be physiologically different from the two large ganglionic cells stained at the border between layers 6 and 7.

Based on the anatomical finding mentioned above, we could expect antidromic spikes from some of the neurons in layer 6. Responses of the selected neurons were graded in amplitude and identified as postsynaptic potential and we could not identify any full-sized antidromic spike. However, two cells which were not selected here had a small action potential with short latency (1.9 and 2.3 ms) which resembled the *m* spike. The positive proof of antidromic responses in other cells could not be provided. The reason we could hardly find antidromic responses might be attributed to the damage of the impaled neurons, which causes the failure to invade the soma. In many cells, spike activities reduced shortly after the impalement, although the resting potential remained unchanged. Even in such cases, postsynaptic potentials could be easily recorded. Another reason we could not observe an antidromic spike might be that the axon of those projecting pear-shaped cells in layer 6 comes

out from the main dendrite at some distance from the soma (Gruberg & Lettvin, 1980). Gruberg and Lettvin (1980) also suggest that the cell-body recording may fail to detect the important signals that can affect the output of the cell. If the projecting cells have axon collaterals which terminate within the optic tectum, NI stimulation may cause postsynaptic potentials in tectal cells through this pathway. We cannot completely deny this possibility, but we do not have such anatomical evidence for projecting pear-shaped cells in layer 6.

Twenty percent of the cases in the study demonstrate multiple cell labelings. This phenomenon has been reported in the turtle retinal neurons (Stewart, 1978), in the rat nigral dopaminergic neurons (Grace & Bunney, 1983), and in the teleostean isthmic neurons (Williams et al., 1983). All these studies use Lucifer yellow as the fluorescent marker. It has also been described in the cat spinal cells injected with Procion yellow (Zieglgansberger & Reiter, 1974) and in the frog tectal neurons intracellularly stained with cobaltic lysine (Matsumoto et al., 1986). In all cases, the tissue between the injected cell and its neighboring marked cells is completely free of the marker substance. It has been termed "dye-coupling," which occurs primarily but not exclusively between cells known to be electrotonically coupled (Stewart, 1978). Extracellular but not intracellular recordings have provided some evidence for the existence of electrical coupling by demonstrating synchronized action potentials in the neurons known to have dye-coupling (Grace & Bunney, 1983; Williams et al., 1983).

Taken together, NI influences on tectal function, as described in this paper, and isthmotectal circuitry may modify the notion of tectal columns (Szőkely, 1973; Szőkely & Lázár, 1976) as relatively autonomous functional units (Matsumoto et al., 1986), and it should also be incorporated into large-scale models of visuomotor coordination in amphibians (Arbib, 1987).

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