

Postsynaptic Potentials and Morphological Features of Tectal Cells in Homing Pigeons*

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Abstract In this study postsynaptic potentials responding to the electrical stimulation of the optic nerve and the nucleus isthmi pars parvocellularis (Ipc) were intracellularly recorded from 86 tectal cells in pigeons, 13 of which were marked with Lucifer yellow (LY). According to types of postsynaptic potentials and their succession, the postsynaptic responses could be classified into four types: excitatory postsynaptic potential (EPSP)—inhibitory postsynaptic potential (IPSP) sequence (EI type), E, I and IE types. Among them, E or EI responses were mainly produced to the optic nerve stimulation, while the electrical stimulation of the Ipc elicited I responses in more than half the recorded cells. LY-marked tectal cells included five ganglionic cells, four pyramidal cells, two bipolar cells and two stellate cells. The ganglionic cells were mainly located in tectal layers III and IV, whereas the others were in layer II. It appears from both intracellular recordings and fluorescent markings that there is some correlation between the morphological features and the postsynaptic response types of tectal cells.

Keywords: homing pigeon, nucleus isthmi, tectum, postsynaptic potentials, electrical stimulation, fluorescent marking.

In lower vertebrates, the optic tectum (OT) in the midbrain is an important visual center, to which the nucleus isthmi (NI) is closely related. The NI receives its input from the ipsilateral tectum; the avian NI projects back to the ipsilateral OT^[1], while in amphibians and reptiles the NI sends bilateral projections to tectum^[2-6]. These isthmotectal projections are in register with the retinotectal projections.

Both extracellular recordings and markings have shown that the amphibian NI, the nucleus isthmi pars magnocellularis (Imc) in reptiles, and the two subdivisions of the avian NI (Ipc and Imc) are all visual centers, and their cells could be excited by the tectoisthmic fibers^[7-11] and they might use acetylcholine (ACh) as neurotransmitter^[12-15]. Histochemical studies^[14,15] have suggested that the isthmotectal pathway could also be cholinergic^[16-18]. However, electrolytic lesions of the NI disinhibited tectal cells^[19]. Recently Wang and Matsumoto^[20] have indicated that the amphibian NI could directly excite ganglionic cells, and exert stronger inhibition in other cells within the OT. In order to further reveal the functional nature of the isthmotectal projection, the present study investigates postsynaptic potentials and cellular morphologies of the pigeon tectal cells responding to the electrical stimulation of the Ipc by using both intracellular recordings and marking techniques.

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1 Materials and Methods

The experiments were performed on adult homing pigeons (*Columba livia*), 300–500 g body weight. The pigeons were anesthetized with intramuscularly injected pentobarbital sodium (initial dose 0.18 mL/100 g b.w.), artificially ventilated using unidirectional airflow of 95% O₂–5% CO₂ mixture through a trachea cannula, and body temperature maintained at 41°C by a heating pad, and then paralyzed with intramuscular injections of 1% gallamine triethiodide (initial dose 0.02–0.05 mL/100 g b.w.). The left optic tectum and the right optic papilla were surgically exposed. A tungsten bipolar electrode (0.5 mm long exposed tip, 1 mm in interpolar distance) was placed on the papilla, and another tungsten bipolar (0.4 mm between poles) inserted into the Ipc according to its stereotaxic coordinates^[21] (Fig. 1). For electrical stimulation, single rectangular pulses of 0.1–1.5 mA in intensity, 50–100 μs duration and 1 Hz in frequency were delivered. By the end of experiments, negative DC-current (200 μA, 30–45 s) was passed to make a lesion for histologically verification of the electrode positions.

Intracellular recordings were obtained with a micropipette filled with 3 mol/L potassium acetate (0.5–1.0 μm in tip diameter, 20–60 MΩ in impedance). Intracellular responses were fed into a DC preamplifier and then either displayed on an oscilloscope or sent to a signal averager. The averaged output was plotted with an X-Y plotter. In a few of experiments, micropipettes were filled with 5% Lucifer yellow (LY), which was injected into the impaled cells by negative current (2–5 nA, 2–5 min) after recording postsynaptic potentials. After 4 h survival, the brain was removed and fixed in a mixture (pH 7.4) of 3% formaldehyde and 3% glutaraldehyde, and then immersed in 30% sucrose overnight. Frozen sections were cut at 100 μm in thickness, histologically processed, and then photographed with a fluorescent microscope. The morphologies of the marked cells were traced based on the photos of serial sections. After being photographed, the sections were counterstained with cresyl violet to distinguish the tectal lamination.

2 Experimental Results

2.1 Postsynaptic Potentials of Tectal Cells

In 31 pigeons, 80% of the impaled cells (86 cells) responded to the electrical stimulation of both the optic nerve and the Ipc, and 20% of cells responded only to the optic nerve stimulation and they were omitted for analysis. The resting membrane potentials ranged from –20 mV to –70 mV, with an average of -40 ± 15 mV. Generally speaking, tectal cells in the superficial layers have smaller membrane potentials in amplitude (–20 to –30 mV), while those in deep layers have larger membrane potentials (–40 to –70 mV). Electrical lesions made in 31 pigeons indicated that the stimulating electrode was all positioned within the Ipc, and the reference electrode also placed in the nucleus in most cases (Fig. 1).

Following the optic nerve stimulation, excitatory postsynaptic potential (EPSP)—inhibitory postsynaptic potential (IPSP) sequence (EI type) was produced in 49 tectal cells (57%), single EPSP with occasionally 1–2 action potentials (E type) in

21 cells (24%), single IPSP (I type) in 15 cells (18%) and IPSP—EPSP responses (IE type) in 1 cell (1%). By considering the first postsynaptic potential, the optic nerve stimulation evoked excitatory responses in 70 tectal cells (81%) and inhibitory ones in 16 cells (19%). On the contrary, the Ipc stimulation elicited inhibitory responses in more than half the recorded cells: I type in 43 cells (50%) (Fig. 2, A) and IE type in 2 cells (2%); E type responses were produced in 19 cells (22%) and EI type in 22 cells (26%) (Fig. 2, B).

2.2 Latencies of Postsynaptic responses

Fig. 4 illustrates the distribution of latencies of postsynaptic potentials (the first potentials) of tectal cells. The latencies of EPSPs evoked by the optic nerve stimulation were distributed between 1–8 ms with a peak at 2.5 ms, while those of IPSPs elicited by the stimulation were longer than 6 ms in most cells without a peak (Fig. 3). IPSPs of shorter latencies (2–3 ms) suggested direct inhibitory input from the retina to the tectum.

The latencies of EPSPs evoked by the Ipc stimulation were distributed between 1–5 ms with a peak at 1.5 ms. The IPSP latencies had a similar distribution (Fig. 3). However, for EPSPs 82% of latencies were 1–2 ms, indicating that the excitatory Ipc-tectal projection is mainly monosynaptic transmission; while for IPSPs 64% of latencies were longer than 2 ms, implying that the inhibitory Ipc-tectal fibers are mostly of disynaptic or multisynaptic nature. This is also supported by the facts that most EPSPs with shorter latencies (shorter than 3 ms) could follow the electrical stimulation of 45–80 Hz, whereas most IPSPs could not follow that higher than 6–10 Hz, with an exception of shorter latency IPSPs recorded in the superficial layers, which could follow the electrical stimulation of 45 Hz.

2.3 Morphologies of the Marked Cells

Among 13 LY-marked tectal cells are 4 pyramidal, 2 bipolar, 2 stellate and 5 ganglionic cells (Figs. 4 and 5). The pyramidal cells are localized in tectal layer II. Their somata are pyramidal or fusiform in shape, and an apical dendrite runs through 3–6 sublayers vertically upwards and ramifies to form a bush of branches in the area where the optic fibers terminate. They usually have 1–2 basal dendrites which sprout out some branches going laterally and ventrally. Bipolar and stellate

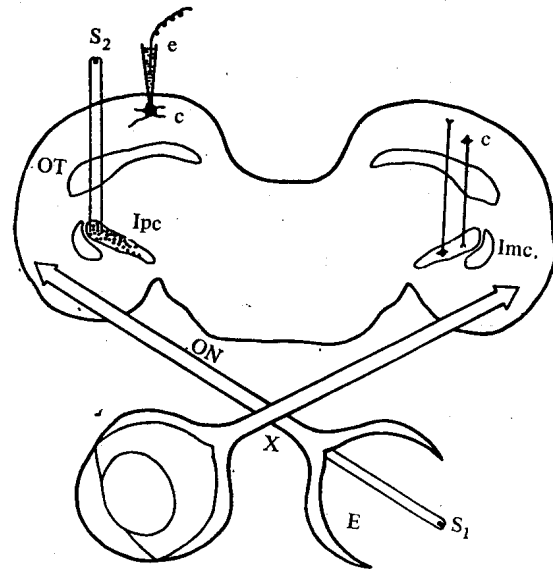


Fig. 1. Experimental set-up. E, Pigeon's eye; Imc, nucleus isthmi pars magnocellularis; Ipc, nucleus isthmi pars parvocellularis; ON, optic nerve; OT, optic tectum; X, chiasm; C, cell body; e, intracellular recording electrode; S₁, stimulating electrode on the optic papilla; S₂, stimulating electrode in Ipc; Black spots within the Ipc show the positions of stimulating electrodes in 31 pigeons.

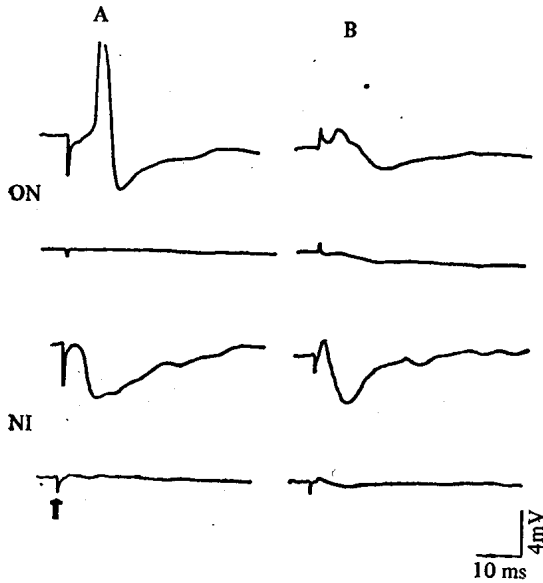


Fig. 2. Postsynaptic responses of tectal cells (A, B) to the optic nerve stimulation (ON) and the Ipc stimulation (NI). Lower traces under each postsynaptic potential show field potentials recorded just outside of the impaled cell. Cell A produced EPSP-IPSP sequence (EI type) responses to the ON stimulation, and IPSP responses (I type) to the NI stimulation. Cell B evoked EI type responses to the ON and NI stimulations. Arrow indicates electrical stimulation.

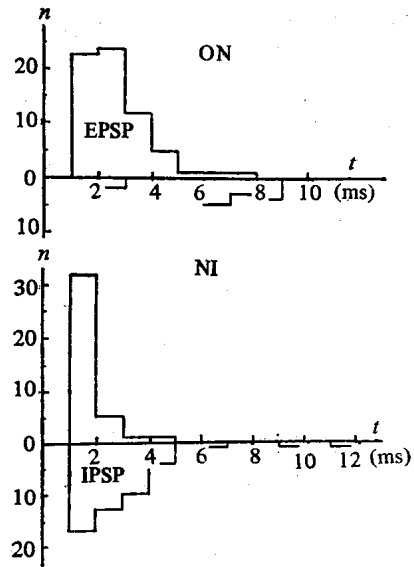


Fig. 3. Histograms of latencies of postsynaptic potentials produced by tectal cells responding to the optic nerve stimulation (ON) and the Ipc stimulation (NI). Abscissa t represents latencies (ms) of postsynaptic potentials, and ordinate n represents the number of cells. Upward histograms are the distribution of EPSP latencies, and downward histograms are the distribution of IPSP latencies.

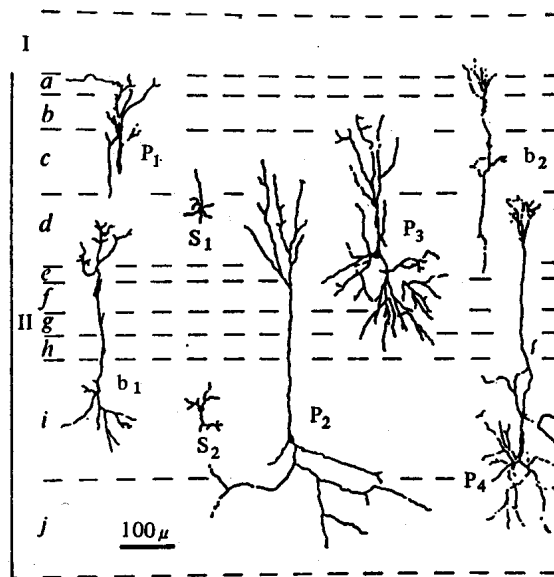


Fig. 4. Morphologies of Lucifer yellow-marked tectal cells and their distribution in tectal layers. I and II, two upper layers of tectum; $a-j$, sublayers of layer II; b_1 and b_2 , bipolar cells; P_1-P_4 , pyramidal cells; S_1 and S_2 , stellate cells. Postsynaptic potentials corresponding to these cells are shown in Fig. 6.

cells are all localized in layer II. The bipolar cells have somata smaller than $10\ \mu\text{m}$ in diameter and a vertically ascending and a descending primary dendrite, which forms a bush of terminal branches.

The stellate cells have spherical somata and short dendrites which radiate to form a much smaller dendritic field. The ganglionic cells are distributed in tectal layers II–IV, and have triangular, fusiform or rod-shaped somata ($20\text{--}30\ \mu\text{m}$), which send out 2–6 primary dendrites usually with a few branches forming broader dendritic fields ($500\text{--}1000\ \mu\text{m}$). Cells g_5 in layer II sends a thicker axon (about $5\ \mu\text{m}$ in diameter) which sprouts out two collaterals in layer III and travels

rostroventrally through layers IV and V to participate in the efferent pathway from the tectum. Cell g_3 has dendrites which mainly spread out horizontally and form a dendritic field with the longest extension of about $1800\ \mu\text{m}$. Cell g_1 possesses radial dendrites and an axon-like process which gives a collateral and then runs horizontally.

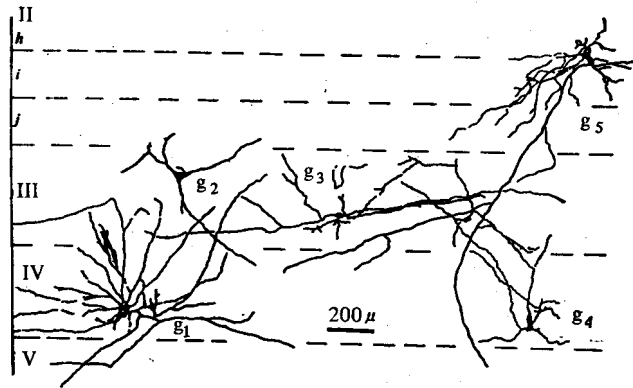


Fig. 5. Morphologies of Lucifer yellow-marked tectal cells and their distribution in tectal layers. *h–j*, Sublayers of tectal layer II (continued from Fig. 4); III–V, lower three layers of tectum; $g_1\text{--}g_5$, ganglionic cells. Postsynaptic potentials corresponding to these cells are shown in Fig. 6.

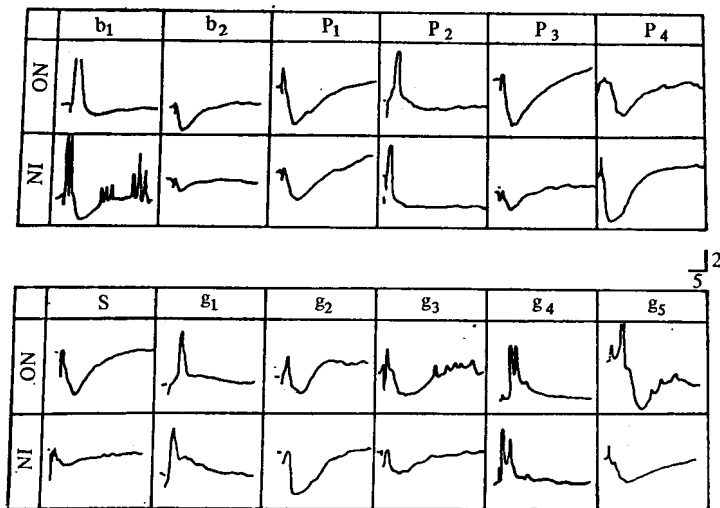


Fig. 6. Postsynaptic potentials produced by tectal cells responding to the optic nerve stimulation (ON) and the Ipc stimulation (NI). After being intracellularly recorded, the cells were labeled with Lucifer yellow. The letters and numerals correspond to those in Figs. 4 and 5. S represents S_1 or S_2 . Scale: 5 ms and 2 mV.

2.4 Correlation Between Cellular Morphologies and Intracellular Responses

Although the data obtained from 13 cells are far from sufficient to conclude the correlation between cellular morphologies and intracellular responses, we can still get some hints from them. Comparisons of Fig. 4 and Fig. 5 with Fig. 6 show that some cells with similar morphologies have type-identical postsynaptic responses. For example, the two stellate cells S_1 and S_2 in Fig. 4 gave identical I type responses, and the optic nerve stimulation evoked stronger responses than the Ipc stimulation did (Fig. 6,s). The ganglionic cells g_1 and g_4 localized in tectal layer IV have rod-shaped somata and responded to both stimulations by EPSPs and in a similar strength. The ganglionic cells g_2 and g_3 in layer III as well as g_5 in layer II produced EI type responses to the optic nerve stimulation, and I type responses to the Ipc stimulation.

Based on postsynaptic potentials, cellular morphologies, latencies and electrical stimulation follow-up frequencies, it appears that the avian Ipc could directly excite a proportion of ganglionic, pyramidal and bipolar cells, and exert strong inhibition on the other tectal cells receiving input from the Ipc. It is shown from both postsynaptic potentials and cellular morphologies that at least the two stellate cells recorded could be inhibitory interneurons.

3 Discussion

About 80% of tectal cells impaled in the present study responded to both optic nerve stimulation and Ipc stimulation, and other 20% of tectal cells responded only to the optic nerve stimulation. It is indicated that the avian Ipc is an important source of afferents to the tectum.

In birds the Ipc exerts inhibitory and excitatory effects on tectal cells. This is similar to the results obtained on bullfrogs^[20]. It is known that the electrical stimulation of the Ipc released glycine from the pigeon tectum^[22,23]. Microiontophoretically applied glycine could strongly inhibit the activity of tectal cells^[24]. These results suggest that the Ipc-tectal pathway in pigeons contains at least a part of fibers which use glycine as an inhibitory transmitter. Similarly, γ -aminobutyric acid (GABA) might be another inhibitory transmitter within this pathway^[1,24,25]. Excitatory effects of the Ipc on tectal cells could be produced at the cholinergic synapses^[14]. Our previous study has shown that acetylcholine could excite tectal cells by means of muscarinic receptors^[18].

The tectal cells that were stained with the fluorescent dye and produced EPSPs could be grouped into two types: cells b_1 , P_1 and P_2 in Fig. 5 spread their dendrites into the optic nerve termination layers (sublayers II_{a-f}), where they directly receive the retinal input. This is supported by latency measurements (about 2.5 ms) and higher frequency (45 Hz) follow-up; cells g_1 — g_5 in Fig. 6 have not dendrites spreading into the retino-recipient layers. Usually, they had a latency period of more than 3.1 ms, and an electrical stimulation follow-up frequency of less than 6 Hz. It appears that these cells make multisynaptic connections with the optic nerve terminals. About 20% of the recorded cells receive their inhibitory input from the

retina. A few of them have shorter latencies and follow 45 Hz stimulation, suggesting monosynaptic inhibition^[26]; the others have longer latencies (more than 6 ms) and could not follow more than 6 Hz stimulation, appearing disynaptic or multisynaptic input from the retina.

Cells b_2 , P_1 , P_3 , and S_1 producing IPSP responses to the Ipc stimulation spread their dendrites into the Ipc-tectal projection region (sublayers II_{b-d}), and possibly received direct inhibitory input from the Ipc. Their latencies were all less than 4 ms, and cell P_1 tested could follow 45 Hz stimulation. Cells s_2 , g_2 , g_3 and g_5 producing IPSP responses to the Ipc stimulation were localized within the lower part of layer II and layer III, without dendrites in sublayers II_{b-d} . However, their shorter latencies (2.8—4.2 ms) imply their receiving monosynaptic input. The other cells evoked excitatory responses to the Ipc stimulation. Cells b_1 , P_2 and P_4 all spread their dendrites into the Ipc-tectal termination area (sublayers II_{b-d}), and probably receive direct Ipc input. In fact, they had very short latencies (1—2 ms), and could follow 45 Hz stimulation (P_2 and P_4). Cells g_1 and g_4 were localized in the lower part of layer IV, without dendrites being seen to enter into sublayers II_{b-d} . However, it appears from latencies (1.3 ms) and stimulation follow-up ability that they also directly receive the Ipc input. There are two possibilities to explain this disagreement: the fluorescent dye could not stain their dendrites in sublayers II_{b-d} , or some of the Ipc-tectal fibers could also terminate in the lower part of layer II and layer III.

The tectal cells in birds could be morphologically classified into four types, i.e. pyramidal, ganglionic, stellate and bipolar cells^[26]. The cells marked in the present study include all the types, with cells of the first two types being proportionally more numerous. It is apparent that every type of tectal cells in birds all receive the Ipc input. Hardy *et al.*^[26] identified all the marked ganglionic cells as efferents, while only one of the ganglionic cells (Fig. 6, g_5) showed an efferent fiber. It may be because of difference between Lucifer yellow and horseradish peroxidase in labeling morphological details. It is noteworthy that some of the 4 cells marked in tectal layer II_j (Fig. 5) may project to the Ipc. If it is true, a neuronal circuitry is formed between the tectum and the Ipc. Of course, stellate cell S_2 in layer II_j and S_1 in II_d may be interneurons, which produced IPSP responses to the electrical stimulation of the optic nerve and the Ipc. However, the two stellate cells found by Hardy *et al.* (1985) responding to the optic nerve stimulation only evoked EPSPs^[26]. It seems that stellate cells could be functionally classified into distinct groups. Therefore, determining the correlation between postsynaptic responses and morphological features must be based on sufficient data of intracellular recordings and dye-stainings.

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