

Tectal afferents monosynaptically activate neurons in the pigeon isthmo-optic nucleus

Wen-Chang Li, Jing Hu and Shu-Rong Wang*

Laboratory for Visual Information Processing, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

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ABSTRACT: Postsynaptic responses of 105 neurons in brain slices were intracellularly recorded from the isthmo-optic nucleus (ION) in pigeons, and 18 of these neurons were labeled with Lucifer yellow. Excitatory postsynaptic potentials (EPSPs) or spikes were produced in 93 cells, inhibitory postsynaptic potentials (IPSPs) in 10 cells, and EPSPs followed by IPSPs in two cells following electrical stimulation of the tecto-isthmo-optic tract. The EPSPs occurred in an all-or-none fashion, with short latencies (1.3 ± 0.6 ms). Repetitive stimulation increased their amplitude and duration, demonstrating that temporal summation was involved. Neurons producing excitatory responses were distributed throughout cellular layers of the nucleus. Pure IPSPs had a latency of 3.9 ± 2.3 ms, and cells that responded in this manner were only distributed in the rostral portion of the nucleus. In the remaining two cells with EPSP-IPSP responses, the latency of excitatory responses was 1.5 ms in one cell and 1.4 ms in the other, and that of inhibitory responses was, respectively, 5.1 and 4.1 ms. Thus, it appeared that excitation was monosynaptic, whereas inhibition may be polysynaptic. Four single injections resulted in dye-coupled labeling, and two pairs of closely apposed cells fired spikes, probably resulting from spatial summation of their excitatory responses. The present study suggests that tectal cells directly activate ION neurons and that tectal fibers contact isthmo-optic neurons in a one-to-one fashion. Taken together with previous studies, it appears that the entire tecto-ION-retinal pathway is excitatory. © 1999 Elsevier Science Inc.

KEY WORDS: Brain slice, Intracellular recording, Isthmo-optic nucleus, Lucifer yellow, Optic tectum, Pigeon.

INTRODUCTION

The isthmo-optic nucleus (ION) in ground-feeding birds such as pigeons is the most conspicuous and extensively studied centrifugal or retinopetal structure in vertebrates [19,31]. This nucleus and its surrounding region, the ectopic ION, are located in the caudal midbrain, dorsomedial to the optic tectum. It has been shown that the ION receives afferents primarily from neurons in the ipsilateral tectum [7,23,35,38] and projects to the contralateral retina [36,37,39], terminating in either convergent or divergent modes on amacrine cells and/or displaced ganglion cells [4,10,21,28,39]. The pathway from tectum to ION to retina suggests that the tectum can modulate retinal activity.

Several electrophysiological studies have been devoted to the receptive field properties of ION neurons [16,25,34] and to the effects of electrical stimulation of the retinopetal pathway on visual properties of retinal ganglion cells. Stimulation of the isthmo-optic tract or ION enhances visual responses of retinal ganglion cells, probably resulting from facilitation of the excitatory receptive field center or from disinhibition of the surrounding inhibitory receptive field [26,32]. Visual enhancement or facilitation could be explained by the finding that retinopetal input excites amacrine cells, which in turn activate retinal ganglion cells [3,20,33]. Recently, we studied the effects of ION blockade by lidocaine on visual responses of tectal cells and found that this nucleus strongly influences tectal activity via the retina in pigeons [17].

Although a number of neuroanatomical, immunohistochemical and electrophysiological studies have been focused on the retinopetal system [31], our knowledge about the physiology of the tecto-isthmo-optic pathway is still lacking. An early study reported that most of the ION can be activated by electrical stimulation of the lateral tectum, producing spike-like field potentials [14]. However, gamma-aminobutyric acid (GABA)-immunoreactive fiber terminals on retinopetal neurons are numerous, and most of these may originate in the tectum [24]. This implies that some tectal afferents to the ION might be inhibitory. Therefore, the present study was undertaken to elucidate further the physiology of the tecto-isthmo-optic pathway by applying intracellular recording and staining procedures to brain slice preparations of pigeons.

MATERIALS AND METHODS

The experiments were performed on brain slices of 10 adult pigeons (*Columba livia*), 310–480 g body weight, following guidelines regarding the use of animals in neuroscience research approved by the Society for Neuroscience. The animals were anesthetized with an injection of ketamine hydrochloride (30 mg/100 g b.w.) and then decapitated. The brain was immediately removed from the skull and washed in ice-cold Krebs-Ringer solution containing (in mM) NaCl, 124; KCl, 5; CaCl₂, 2; MgSO₄, 2; KH₂PO₄, 1.25; NaHCO₃, 26; glucose, 10 [13], oxygenated with a mixture of 95% O₂ and 5% CO₂. Midbrain tissue blocks including the ION and the tecto-isthmo-optic tract (TIO) were blocked and glued on the stage of a Vibroslice (Campden Instruments 752M, UK). Slices containing the ION and TIO were sectioned

* Address for correspondence: Dr. Shu-Rong Wang, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun, Beijing 100101, China. E-mail: wangsr@mimi.cnc.ac.cn

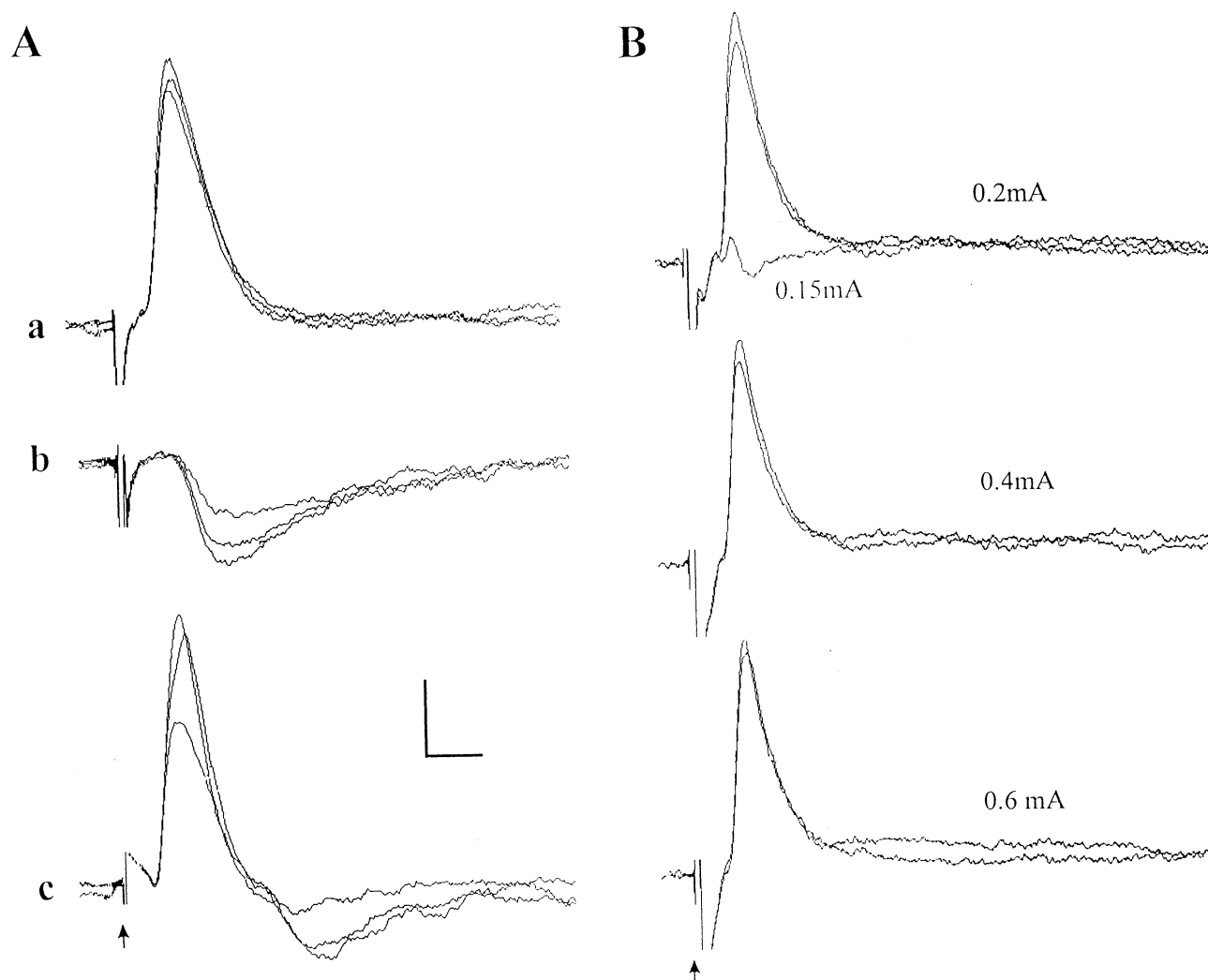


FIG. 1. Postsynaptic responses of isthmo-optic neurons to electrical stimulation of the tecto-isthmo-optic tract. (A) Following tract stimulation, most isthmo-optic neurons produced excitatory postsynaptic potentials (a), and a small proportion of neurons generated inhibitory postsynaptic potentials (b) or EPSPs followed by IPSPs (c). (B) Isthmo-optic neurons produced all-or-none responses to tract stimulation, whose amplitudes were not dependent on intensity of electrical stimulation (0.2–0.6 mA). Arrows point to artifacts of electrical stimulation. Three (A) or two (B) sweeps were superimposed. Scales: 2 mV, 2 ms.

horizontally at $350\ \mu\text{m}$ in thickness and then transferred from a storage container into the recording chamber (BSC-HT, Medical System Corp., Greenvale, NY, USA) perfused with Krebs-Ringer solution bubbled with a mixture of 95% O_2 and 5% CO_2 . The slices were incubated at 30°C for 60 min.

Under an operating microscope, the TIO was seen merging anterolaterally with the ION. A bipolar tungsten electrode with poles $400\ \mu\text{m}$ apart was placed across the TIO and about 1.5 mm distant from the ION. Rectangular pulses of $50\text{--}150\ \mu\text{s}$ in duration and $150\text{--}600\ \mu\text{A}$ in intensity were delivered for electrical stimulation. For intracellular recording and staining, a micropipette filled with either 3 M of potassium acetate or aqueous solution of Lucifer yellow (5%, dilithium salt, Sigma, St. Louis, MO, USA) was used. The electrode tip was broken to $0.5\text{--}1\ \mu\text{m}$ by touching it against the edge of a glass slide with a micromanipulator under a microscope. The micropipette was advanced into the ION in slices under visual control. Isthmo-optic neurons were impaled by

applying brief positive current pulses (4 nA in intensity, 0.3 s in duration). Intracellular impalement was signaled by a sudden direct current (DC) drop of $20\text{--}60\ \text{mV}$. Postsynaptic potentials evoked by tract stimulation were amplified (WPI Intra 767, Sarasota, FL, USA), stored on magnetic tapes (TEAC RD-135T Data Recorder, TEAC Corp. Tokyo, Japan) and then analyzed. In some experiments, the dye was injected by passing a negative current of $2\text{--}4\ \text{nA}$ through the electrode for $2\text{--}10\ \text{min}$. In most cases, only one injection of Lucifer yellow was made in each slice; otherwise, two injections were made in sites about $1000\ \mu\text{m}$ apart. After $0.5\text{--}2\ \text{h}$ of survival, slices were removed from the recording chamber, fixed in 8% paraformaldehyde and kept in a refrigerator overnight. The slices were rinsed with physiological saline and then placed in 100% dimethylsulfoxide (DMSO) for 20 min [12]. The DMSO-mounted slices containing Lucifer-yellow-marked ION cells were coverslipped and observed or photographed with a fluorescence microscope.

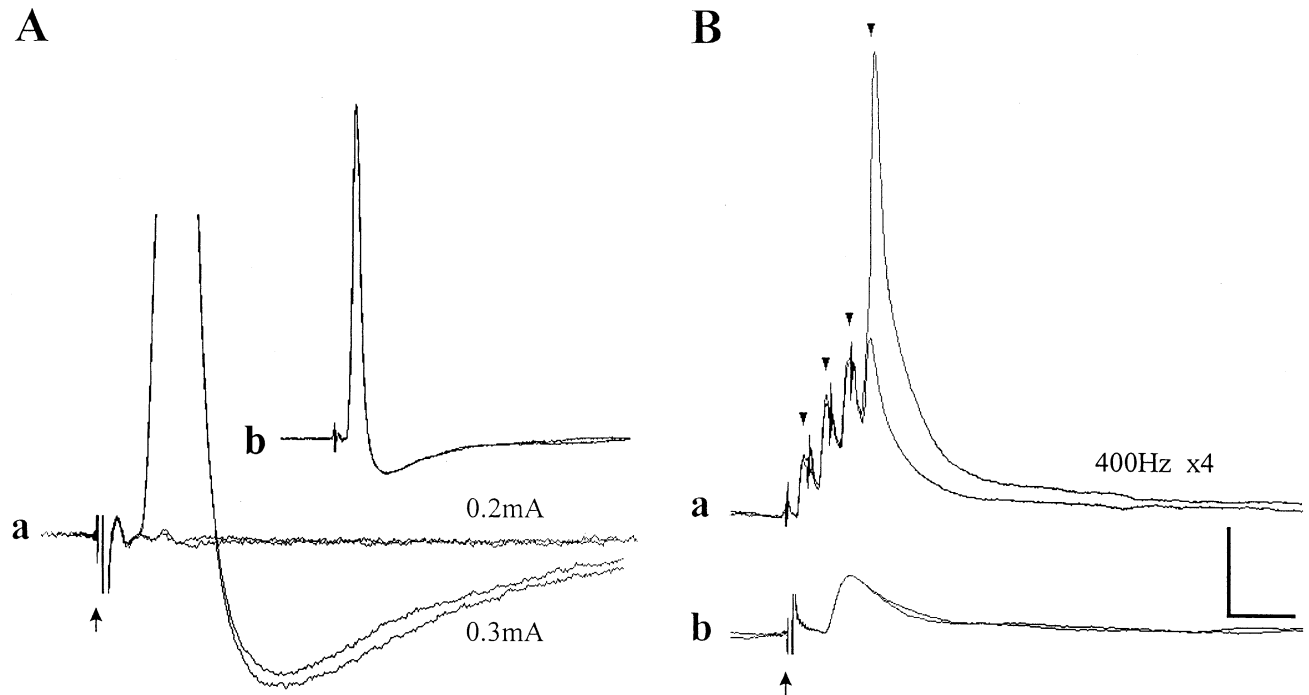


FIG. 2. Spike firings elicited by tract stimulation in some ION cells depended on stimulation intensity or repetitive stimulations. (A) Increasing stimulation intensity from 0.2 to 0.3 mA evoked a spike in an isthmo-optic neuron (a). Inset b is a full profile of the spike shown in a. (B) EPSPs of an isthmo-optic neuron were superimposed and thus increased in amplitude to finally produce a spike when four stimulations were applied (a). Frequency (400 Hz) and repetition (4 stimulations) are shown above traces. Traces in b depict superimposed EPSPs generated by two stimulations. Arrows point to electrical stimulation artifacts; arrowheads point to EPSPs and spikes elicited by stimulations. Two sweeps were superimposed. Scales: 3 mV, 2 ms in Aa; 12 mV, 7 ms in Ab and Ba; and 12 mV, 2 ms in Bb.

RESULTS

Postsynaptic responses of 105 isthmo-optic neurons to tract stimulation were intracellularly recorded, and 18 of these were labeled with Lucifer yellow to show their morphological features. Their membrane potentials ranged from -20 to -62 mV, with an average value of -43 mV.

Following electrical stimulation of TIO, 93 of 105 cells produced excitatory postsynaptic potentials (EPSPs) (65%) or spikes (35%). These EPSPs rose and fell rapidly, with an average duration of 4.4 ± 1.9 ms (mean \pm SD, $n = 79$), amplitude of 3.2 ± 1.8 mV and latency of 1.3 ± 0.6 ms. As an example, excitatory responses in Fig. 1Aa were recorded from the cell shown in Fig. 3E₆. This cell was located in the cellular layer of the caudal rim and classified as a multipolar cell [18]. Six primary dendrites bearing numerous varicosities and generally branching two to three times radiated from the cell body. Inhibitory postsynaptic potentials (IPSPs) were evoked in 10 cells following the TIO stimulation. Figure 1Ab shows IPSPs recorded from the cell whose morphology is drawn in Fig. 3I₂. It was located in the rostromedial cell layer, and its spindle-shaped perikaryon issued two dendrites oriented internally and an axon from the opposite pole, which traveled in a tortuous way to the isthmo-optic tract. The IPSPs recorded from these cells had an average latency of 3.9 ± 2.3 ms ($n = 10$), duration of 10.6 ± 2.2 ms and amplitude of 2.2 ± 0.8 mV. The remaining two cells produced spike-like EPSPs followed by IPSPs. The latency of EPSP was 1.5 ms, and that of IPSP was 5.1 ms in one cell; the respective parameters of EPSP and IPSP were 1.4 ms and 4.1 ms in the other. The EPSP-IPSP sequence shown in Fig. 1Ac was recorded from the cell whose morphology

was depicted in Fig. 3E₁. It was located in the caudolateral cell layer and gave rise to a primary dendrite with several branches spreading internally and an axon traveling across the nucleus to participate in the isthmo-optic tract.

Sixty-five percent of the examined ION cells were excited in all-or-none fashion in response to TIO stimulation. As shown in Fig. 1B, amplitude of spike-like EPSPs did not increase as intensity of electrical stimulation increased from 200 to 400 to 600 μ A. Statistics of EPSP amplitudes evoked by various intensities (200, 300, 400, 500 and 600 μ A) indicated that these responses were not significantly different in amplitude (paired *t*-test, $p > 0.1$, $n = 12$). In the remaining 35% of cells, tract stimulation produced spikes (Fig. 2A). An example of postsynaptic responses of an ION cell to repetitive stimulation is shown in Fig. 2B, indicating that spike-like EPSPs increased in amplitude and in duration as the number of stimuli (2–5 pulses) and/or the frequency (100–600 Hz) of stimulation increased.

Four of 18 (22%) single injections of Lucifer yellow resulted in multiple labelings. These dye-coupled cells were closely apposed (twin cells, Fig. 3T_{1,2}) or up to 70 μ m distant from each other (double cells, Fig. 2D_{1,2}, corresponding to Fig. 4a,b). It was interesting to note that following tract stimulation these twin cells produced spikes, whereas the double cells produced spike-like EPSPs and were thus similar to most ION cells. However, two single cells (Fig. 3E_{1,9}) localized in the lateral ION also fired spikes. Generally speaking, it was very hard to correlate the response type with the morphology of these cells. For example, cells E₆ and E₇ in Fig. 3 were morphologically different, but they both produced EPSPs following tract stimulation; conversely, cells

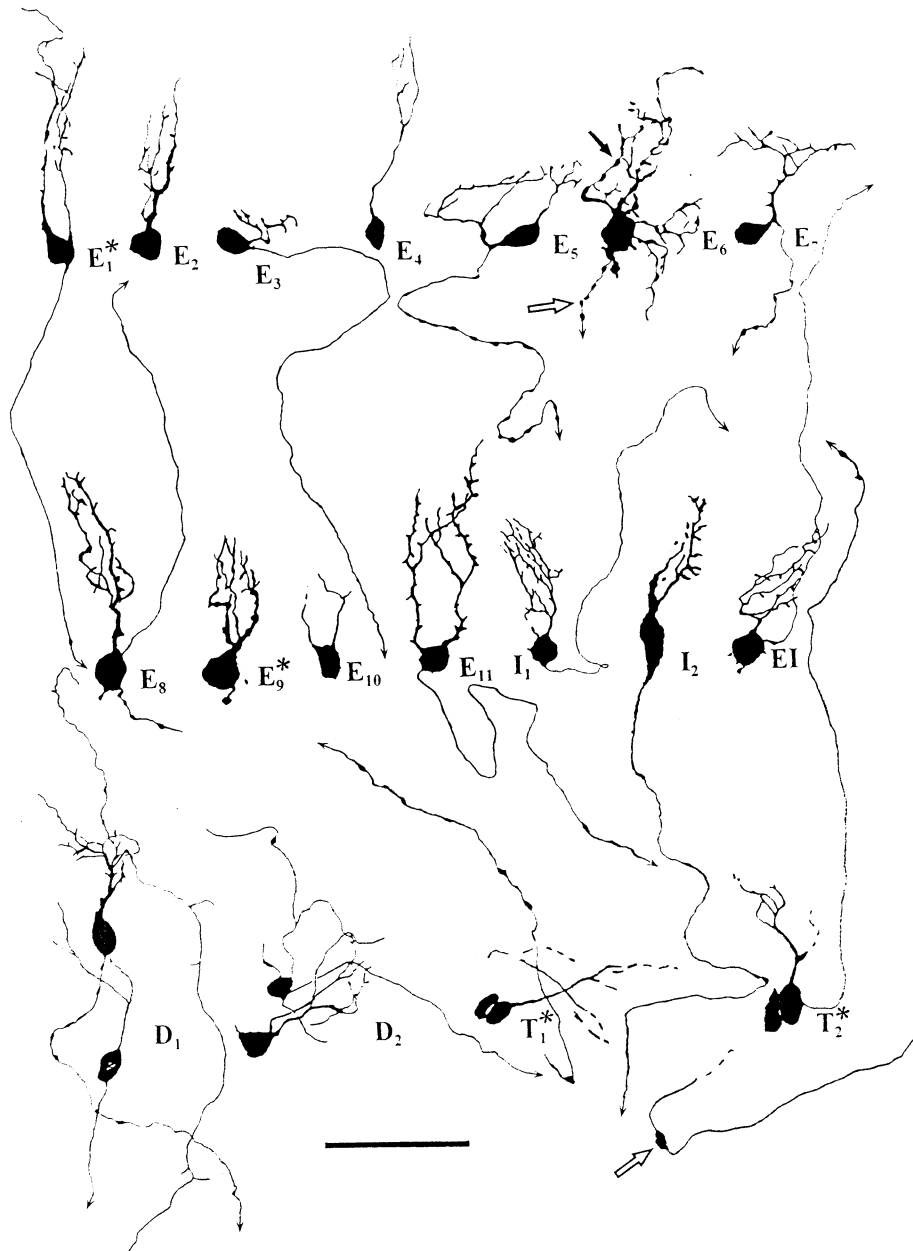


FIG. 3. Morphological reconstruction of isthmo-optic neurons labeled with Lucifer yellow after intracellular recordings were made. Abbreviations: E, EI, and I represent isthmo-optic neurons producing excitatory, excitatory-inhibitory, and inhibitory responses, respectively; D, double cells; T, twin cells. Numerals are ordinal numbers, and asterisks mark cells that fire spikes. Thin lines with arrowheads represent axons, solid arrow points to varicosities on dendrites and empty arrows point to varicosities on axons. Photomicrographs of cells D₁, D₂, E₈ and E₁₁ are shown in Fig. 4. Scale: 100 μ m.

E₁₁ and I₁ had similar morphological features, but their response properties were quite different.

DISCUSSION

The present study employed intracellular recording and staining procedures to provide evidence that the neuronal pathway from the optic tectum to the ION in pigeons is primarily, if not exclusively, excitatory. This is in agreement with the finding that

electrical stimulation of the lateral tectum elicited field potentials in the ION, suggesting that the tectum can activate ION cells [14]. This study shows that most ION cells respond in an all-or-none manner to the TIO stimulation. These all-or-none responses resemble those of the principal neurons in the tangential nucleus to vestibular nerve stimulation in brain slices of the chick embryo [30] and those of Purkinje cells to stimulation of climbing fibers in cats [8]. These responses could be explained by one-to-one relation

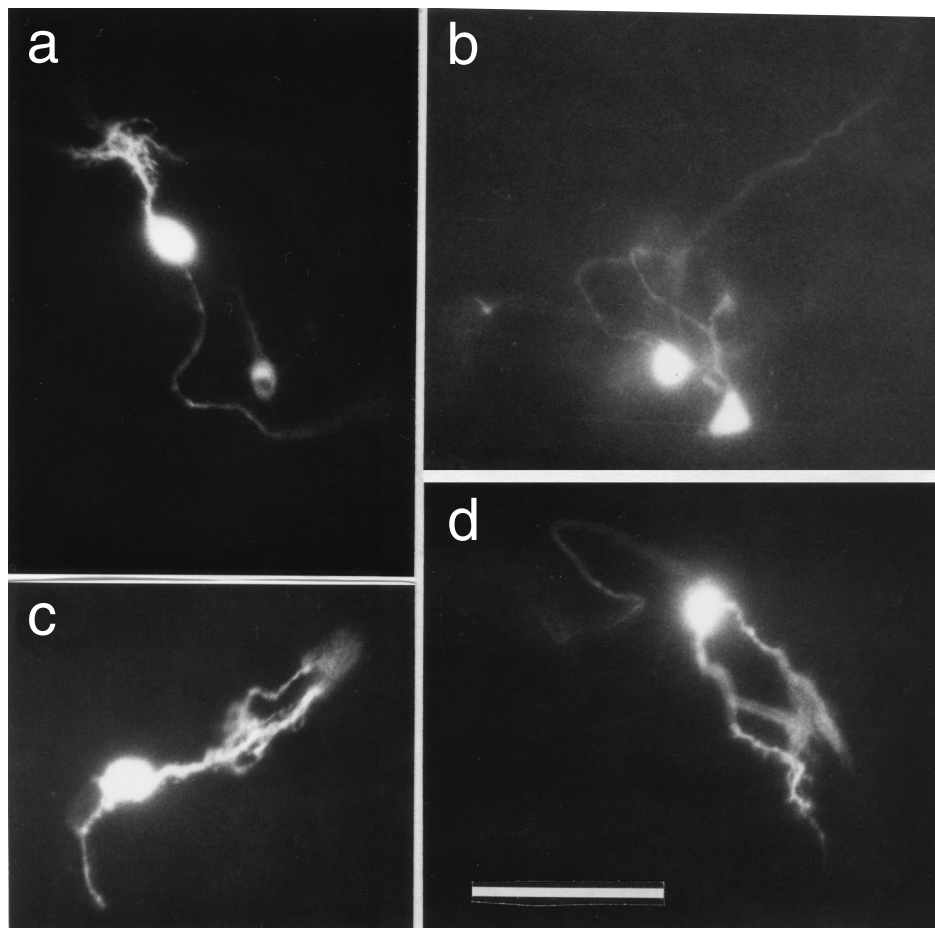


FIG. 4. Photomicrographs showing morphological features of some Lucifer-yellow-labeled isthmo-optic neurons. Cells a–d correspond to cells D₁, D₂, E₈ and E₁₁ in Fig. 3. Note that a and b are double cells, each pair of which was labeled by a single injection of Lucifer yellow. It appears that these cells are dye coupled by dendritic contacts. Scale: 100 μ m.

between afferent fibers and their postsynaptic neurons [8,30]. In fact, the number of tectal neurons projecting to the ION approximates that of ION neurons in birds, suggesting that a single tecto-isthmo-optic fiber may contact a single isthmo-optic neuron [35,38]. Such synapses are predominantly axo-dendritic, and the presynaptic terminals contain round vesicles [1,6]. It is conceivable that repetitive stimulation at high frequencies could result in superimposition of numerous EPSPs due to temporal summation (Fig. 2B). In some cases, stimulation could evoke spikes probably due to spatial summation of EPSPs in dye-coupled cells.

The fact that ION neurons are predominantly excited by the TIO stimulation indicates that most of the massive GABAergic input to retinopetal neurons of ION [24] may originate from some extratectal structures [2] including the mesencephalic and pontine reticular formation [22]. Meanwhile, a small population of GABA-immunoreactive interneurons, whose axons may be highly collateralized, has been described within the ION [24]. Therefore, a small number of ION neurons would be di-synaptically inhibited by intrinsic interneurons.

In conclusion, the present study provides convincing evidence that the tectum activates neurons in the ION. These neurons in turn send a feedback signal to the contralateral retina in birds. Our

preliminary results indicated that tectum-ION transmission can be blocked by a glutamatergic antagonist (Li et al., unpublished data). The retinopetal fibers contain nitric oxide [27] but not GABA [24], and their recipient cells in the retina are immunopositive for aspartate and glutamate, two excitatory neurotransmitter amino acids [33]. The hypothesis that the entire retinopetal pathway is excitatory is also supported by several electrophysiological studies. For example, EPSPs are evoked in the turtle ganglion cells and amacrine cells following activation of retinopetal fibers [20], and responsiveness of retinal ganglion cells is enhanced by activation of the retinopetal system [11,26,32]. On the other hand, visual activity of retinal ganglion cells is suppressed by cooling the ION [29], and tectal activity is reduced by lidocaine blockade of the ION [17]. It is interesting to note that in ground-feeding birds the ION is much more developed than in birds of prey [5,9,36,37], and this nucleus receives its major input from the dorsal retina via tectum but projects primarily to the ventral retina [3,15,39]. In addition, the two parts of the retina may be connected by intrinsic interneurons [17,38]. Local enhancement of the ventral retina by, for example, a predator in the sky and its long-range action on the dorsal retina would result in visual attention switching [3]. Long-range competition among ION cells may also be involved in

attentional selection [34]. This hypothesis needs to be studied further electrophysiologically by reversible blockage of ION or behaviorally by lesioning the nucleus.

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