

Dual actions of isthmic input to tectal neurons in a reptile, *Gekko gekko*

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Abstract

We analyzed postsynaptic potentials and dye-labeled morphology of tectal neurons responding to electrical stimulation of the optic nerve and of the nucleus isthmi in a reptile, *Gekko gekko*, in order to compare with previously reported interactions between the optic tectum and the nucleus isthmi in amphibians and birds. The results indicate that isthmic stimulation exerts inhibitory and excitatory actions on tectal cells, similar to dual isthmotectal actions in amphibians. It appears that dual actions of the isthmotectal pathway in amphibians and reptiles are shared by two subdivisions of the nucleus isthmi in birds. The morphology of tectal cells responding to isthmic stimulation is generally similar to that of tectoisthmic projecting neurons, but they differ particularly in that some tectoisthmic cells bear numerous varicosities whereas cells receiving isthmic afferents do not. Thus, it is likely that at least some tectoisthmic cells may not be in the population of tectal cells that can be affected by isthmic stimulation. Forty-four percent of injections resulted in dye-coupled labeling, suggesting extensive electrical connections between tectal cells in reptiles.

Keywords: Dye-coupling, Electrical stimulation, Intracellular recording, Nucleus isthmi, Optic tectum, Reptile

Introduction

It has been shown that the nucleus isthmi (NI) in the midbrain of lower vertebrates is a visual structure, homologous to the mammalian parabigeminal nucleus. The NI receives input from the ipsilateral tectum and projects back ipsilaterally in teleosts and birds, or bilaterally in amphibians, reptiles, and mammals as well (see reviews: Gruberg, 1983; Wang, 1988). Several electrophysiological studies have indicated that isthmic or parabigeminal neurons can be activated by visual stimulation through the tectoisthmic pathway in teleosts (Williams et al., 1983; Northmore, 1991), in amphibians (Gruberg & Lettvin, 1980; Wang et al., 1981, 1982; Wiggers & Roth, 1991), in reptiles (Wang et al., 1983), in birds (Yan & Wang, 1986; Wang & Frost, 1991), or *via* the tectoparabigeminal pathway in mammals (Sherk, 1978, 1979). These pathways may use acetylcholine as a neurotransmitter (Felix et al., 1985; Wu et al., 1994). On the other hand, isthmotectal fibers exert inhibitory and excitatory actions on tectal cells in amphibians (Wang & Matsumoto, 1990), and may use gamma-aminobutyric acid (GABA) (Pollák et al., 1999) and acetylcholine (Ricciuti & Gruberg, 1985; Fite & Wang, 1986; Wallace et al., 1990) as transmitters, respectively. In birds, this nucleus is divided into two parts, the nucleus isthmi pars parvocellularis (Ipc) and the nucleus isthmi

pars magnocellularis (Imc), and they modulate visual activity in tectal cells in differential ways (Wang et al., 1995a). The former sends GABAergic axons to the tectum, forming an inhibitory feedback route (Felix et al., 1994; Gao et al., 1995), while the latter sends glutamatergic and cholinergic axons that make excitatory feedback synapses on tectal cells (Wang et al., 1995b). Recently, we have obtained strong evidence that these two divisions could differentially modulate the extent and responsiveness of receptive fields of tectal neurons in pigeons (Wang, Y. et al., unpublished data).

Though the reptilian nucleus isthmi is also divided into two juxtaposed parts, Ipc and Imc, they are structurally and functionally independent nuclei, with only Imc being a visual center. The Imc makes reciprocal connections with the ipsilateral tectum by a direct loop, and also by an indirect loop *via* the nucleus profundus mesencephali (Wang et al., 1983, 1989). In view of the fact that reptiles are phylogenetically the oldest terrestrial vertebrates, occupying an evolutionary position between amphibians and birds, the present study was carried out to reveal synaptic responses and morphology of tectal neurons receiving input from the nucleus isthmi pars magnocellularis in reptiles.

Methods

The experiments were performed on 24 adult lizards (*Gekko gekko*) with body length of 11–14 cm (from nose to cloaca), wild-caught from southern China. As described previously (Wang et al., 1983), the animal was lightly immobilized by injecting 0.1 ml of galla-

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mine triethiodide (4%), and the surgical area, wounds, and stereotaxic fixation points were anesthetized with procaine. During experiments, the animal was capable of moving, but periodic application of the anesthetic eliminated all indications of pain or discomfort such as body motion or tail-flip. A mixture of 95% O₂ and 5% CO₂ was continuously passed through a pipette into the animal's mouth to facilitate breathing. The left tectum and NI visible on the brain surface as an oval bulge located in the dorso-lateral tegmentum were surgically exposed, and the meninges overlying both structures removed. Under anesthesia, the right eye was opened and its vitreous body removed. Rectangular pulses of current 50–500 μ A in intensity and 0.1 ms in duration at 1Hz were delivered to the NI through a bipolar tungsten electrode with tip diameter of 30 μ m and tips spaced 300 μ m apart. The optic disk was stimulated by a concentric electrode (500 μ m outside diameter, 200 μ m inside) with identical parameters. The current intensities were always kept at threshold to minimize current spread. For intracellular recording and dye injection, a micropipette (0.5–1.0 μ m tip diameter) filled with either 2 M potassium acetate or 5% Lucifer yellow (CH, dilithium salt, Sigma Chemical Co., St. Louis, MO) was advanced into the tectum. A brief positive pulse of about 20 nA was used to facilitate impaling cells. Intracellular responses and field potentials recorded just outside of the impaled neurons were amplified and then fed into an oscilloscope and a magnetic tape recorder. Data were analyzed off-line by superimposing traces of sweeps on an X–Y plotter. Postsynaptic potentials were obtained by subtracting field potentials from intracellular recordings.

Lucifer yellow dye was injected into the impaled cells with negative current of 2–5 nA for 1–2 min. After 2–6 h survival, the lizard was killed under anesthesia and the brain prepared by immersion in fixative containing 3% formaldehyde and 3% glutaraldehyde for 12 h (Wang & Matsumoto, 1990), followed by soaking in 30% sucrose in phosphate buffer at 4°C overnight. Frozen sections were cut at 80 μ m and mounted, and morphology of tectal cells was observed and photographed with a fluorescence microscope.

Results

Postsynaptic potentials were recorded from 120 tectal cells, among which 113 cells responded to electrical stimulation of both the optic nerve (ON) and the NI, six cells responded only to the ON stimulation, and one cell only to the NI stimulation. These latter seven cells were omitted from our analysis. As shown in Fig. 1, following the ON stimulation, excitatory postsynaptic potential (EPSP) followed by inhibitory postsynaptic potential (IPSP) was produced in 76 tectal cells, IPSP in 16 cells, and IPSP–EPSP in 21 cells. When the NI stimulation was applied, EPSP–IPSP responses were elicited in 47 tectal cells, IPSP in 35 cells, and IPSP–EPSP in 31 cells. If considering only the initial potential observed in each neuron's responses, the ON stimulation evoked excitatory responses in 76 (67.3%) cells and inhibitory responses in 37 (32.7%) cells, whereas the NI stimulation excited 47 (41.6%) cells and inhibited 66 (58.4%) cells.

The latency of excitatory responses of tectal cells to the ON stimulation was 3.58 ± 1.36 ms (mean \pm s.d., $n = 74$), whereas that of inhibitory responses was 6.91 ± 2.26 ms ($n = 36$). The following IPSPs in neurons with EPSP–IPSP responses had mean latency of 8.53 ± 2.44 ms ($n = 73$). In the cases of NI stimulation, postsynaptic responses of tectal cells had average latency of 2.59 ± 1.10 ms ($n = 47$) for excitatory potentials, 5.58 ± 2.01 ms ($n = 66$) for inhibitory potentials, and 7.36 ± 2.18 ms ($n = 42$) for the following IPSPs in tectal cells with EPSP–IPSP responses.

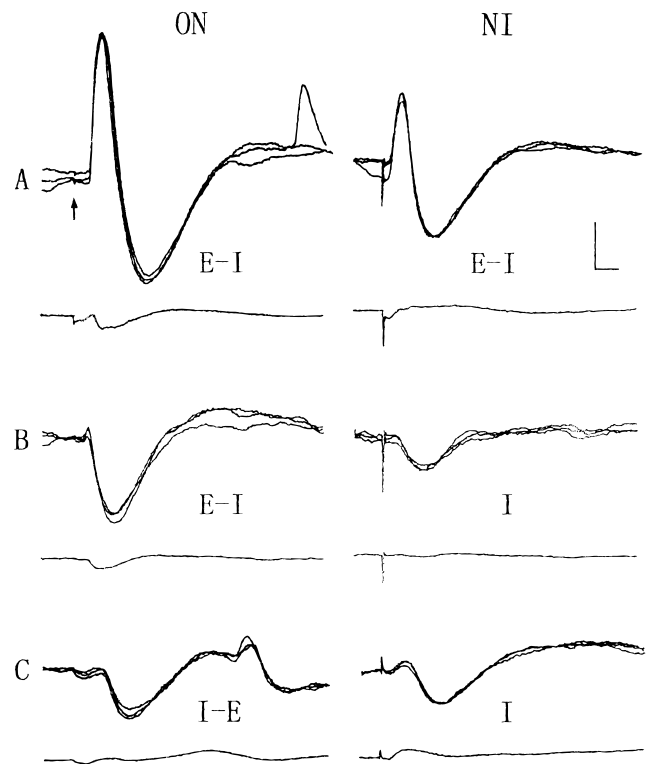


Fig. 1. Postsynaptic potentials of three tectal neurons (A–C) responding to electrical stimulation of the optic nerve (ON) and of the nucleus isthmi (NI). Cell A responded to ON stimulation producing EPSP–IPSP (E–I) responses with rebound excitation, and to NI stimulation with E–I responses; Cell B produced a small and brief EPSP followed by a large and long-lasting IPSP after ON stimulation, and I response to NI stimulation; Cell C elicited I–E responses to ON stimulation and I response to NI stimulation. Three sweeps were superimposed. Traces beneath the intracellular responses are field potentials recorded just outside of the impaled cells. Postsynaptic potentials are obtained by subtracting field potentials from intracellular responses. These cells were dye-labeled and their morphology is depicted in Fig. 2, with corresponding lowercase letters a, b, and c. Arrow points to electrical stimulation artifact. Scales = 5 mV, 10 ms.

Neuronal morphology and laminar distribution were studied in 32 tectal cells labeled by 18 injections, eight of which resulted in multilabeling of up to five tectal cells [Fig. 2 (a, e, f, g, i, j, k, m)]. The distance between somata of multilabeled cells was from 2 μ m to 235 μ m, and their dendrites often intermingled, suggestive of possible interactions *via* dendro-dendritic gap junctions. These coupled cells were usually arranged in radial columns. No axons of the labeled cells could be discerned by the dye, probably due to incomplete dye filling.

The dye-labeled cells could be classified into two types. Neurons of the first type had piriform or pyramidal somata that gave off an apical dendrite pointing toward the pia and sometimes 1–2 short basal dendrites [for example, Fig. 2 (c, o)]. Their dendritic fields were column shaped. Neurons of the second type were fusiform or polygonal, and their dendritic fields were usually elliptical in shape [for example, Fig. 2 (k, l)]. Their dendrites were smooth, with occasional dendrites bearing a few varicosities. This classification reflects the heterogeneity of tectal cells in the lizard (Wang et al., 1983; Yan et al., 1984; Wang et al., 1989). Following the ON stimulation, cells c, l, and p produced inhibitory responses while the others exhibited excitatory responses. Single tectal cells

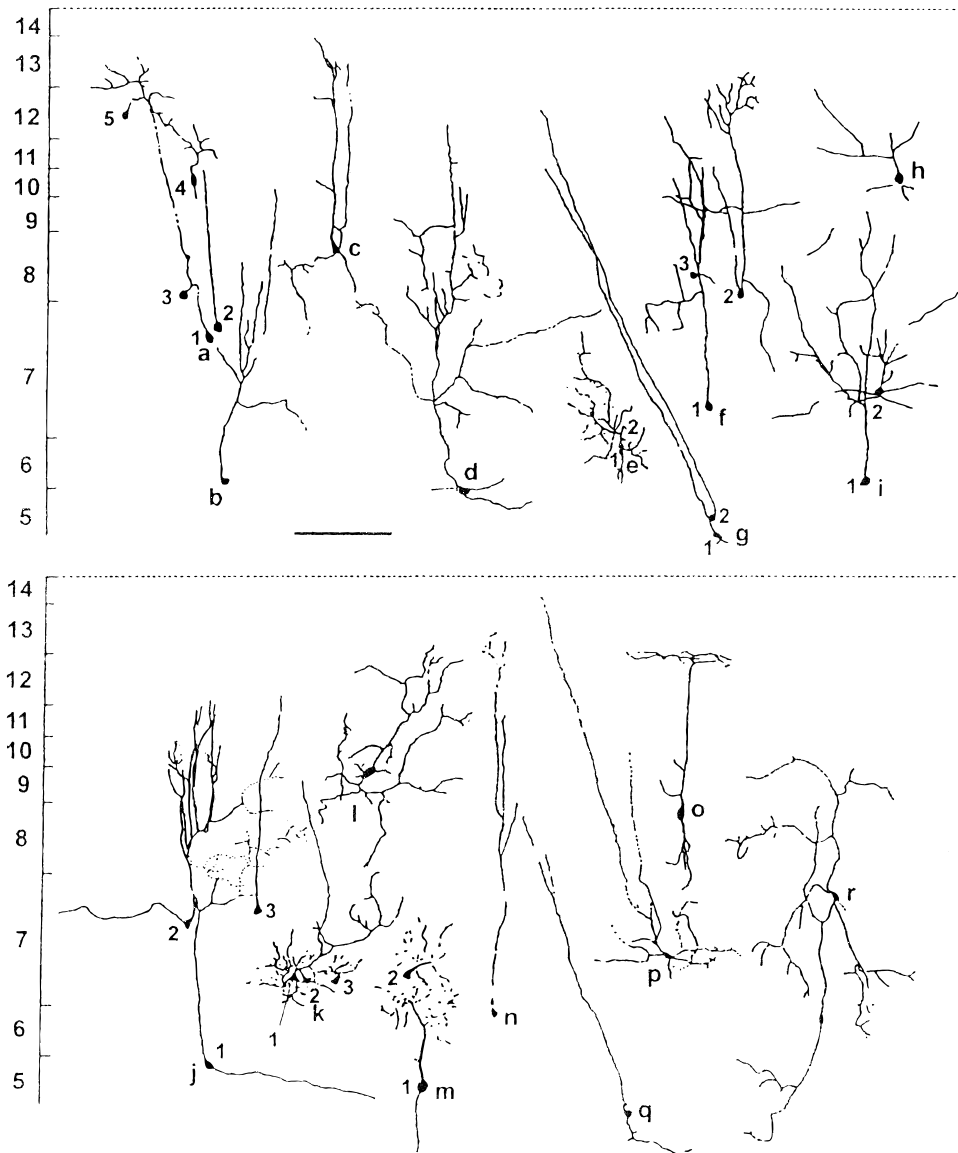


Fig. 2. Morphology and laminar distribution of tectal neurons labeled with Lucifer yellow after intracellular responses were recorded to the optic nerve stimulation and to isthmic stimulation. Postsynaptic potentials of cells a, b, and c are shown in Fig. 1A, 1B, and 1C, respectively. Single tectal cells and groups of dye-coupled cells are arranged with letters a–r, and dye-coupled cells in a group are numbered. Dotted lines indicate discontinuous processes. Numerals on the left represent tectal layers 5–14, and the horizontal broken lines symbolize the pia surface. Scale = 100 μ m.

or groups of coupled cells labeled in 11 of 18 injections [Fig. 2 (b, c, f, h, i, j, l, o, p, q, r)] produced initial inhibitory responses, while seven other cells produced initial excitatory responses to the NI stimulation. In such a small sample of cells, morphology and laminar distribution do not appear to be correlated with type of responses to both stimulations.

Discussion

The present study provides electrophysiological evidence that Imc in reptiles exerts inhibitory and excitatory actions on tectal cells. These dual actions also occur in the isthmotectal system in amphibians (Wang & Matsumoto, 1990). However, incidence of postsynaptic potentials evoked by isthmic stimulation in reptiles is

much higher than that in amphibians (95% vs. 68%, see Wang & Matsumoto, 1990). In both cases, current intensities were always kept at threshold in order to limit current spread within the nucleus. Higher incidence of postsynaptic responses in reptiles may be due to (1) more numerous isthmotectal connections, and/or (2) the existence of two pathways between Imc and tectum, that is, a direct pathway from Imc to tectum and an indirect Imc-nucleus profundus mesencephali-tectum pathway (Wang et al., 1983, 1989). Latency measurement shows that following isthmic stimulation, excitatory potentials are monosynaptic (mean latency = 2.59 ms) while inhibitory potentials may be disynaptic (mean latency = 5.58 ms) or trisynaptic (mean latency = 7.36 ms). This multisynaptic inhibition may arise from intrinsic tectal neurons receiving isthmic input or from profundal neurons. This relay nucleus is

located rostral-dorsal to Imc and likely inhibits tectal activity, as its neurons are known to be immunoreactive for GABA (Bennis et al., 1991). The direct isthmotectal pathway may exert excitatory and inhibitory actions. It has been shown that isthmotectal excitation is exerted by cholinergic fibers acting on postsynaptic muscarinic receptors in the amphibian tectum (Xiao et al., 1999), or by cholinergic and glutamatergic fibers in the pigeon Imc-tectal pathway (Wang et al., 1995b). Isthmotectal inhibition may arise at least in part from intrinsic GABAergic neurons within tectum, supported by the finding that the reptilian tectum contains a dense, laminar distribution of GABA-immunoreactive neurons (Bennis et al., 1991). In amphibians, NI inhibits tectal activity by a GABAergic pathway or intrinsic interneurons via GABA_A receptors (Xiao et al., 1999). In birds, Ipc suppresses tectal activity via GABAergic fibers, with some Ipc cells activating tectal cells by brief cholinergic excitation followed by long-lasting inhibition (Felix et al., 1994; Gao et al., 1995). Therefore, dual actions of the nucleus isthmi in amphibians and in reptiles are shared by the two divisions of the avian NI. These positive and negative feedback pathways probably participate in a "winner-take-all network" to permit the animal to attend to only one of several competing visual targets simultaneously present in the visual field (Serenó & Ulinski, 1987; Wang & Frost, 1991; Wang et al., 1995a). Alternatively, interactions between the retinotectal and the isthmotectal visual map may be used to calculate the three-dimensional trajectories of moving objects (Wiggers & Roth, 1991).

The morphology of tectal cells responding to isthmoc stimulation appears generally to be similar to that of tectal cells projecting to NI (Wang et al., 1983, 1989), but they differ particularly in that dendrites of tectal cells receiving isthmoc input are smooth, while dendritic branches of some tectoisthmoc cells bear numerous varicosities, and in that more tectal cells responding to isthmoc stimulation have polygonal somata and larger dendritic fields in comparison with tectoisthmoc neurons. These differences may imply that tectoisthmoc cells are at least in part not those that receive isthmoc afferents (Gruberg et al., 1994), but the observed differences could also be due to selective labeling or small sample. Dye-coupling has been found in various brain regions in different species, including, for example, NI in teleosts (Williams et al., 1983) and in amphibians (Wu & Wang, 1995; Wiggers, 1998), the optic tectum in amphibians (Wang & Matsumoto, 1990; Wiggers, 1998), the isthmo-optic nucleus in birds (Li & Wang, 1999), and the striatum, neocortex, and hippocampus in rats (Peinado et al., 1993; Onn & Grace, 1994; Velazquez et al., 1997). Dye-coupling has been suggested to be indicative of electrotonic connections (Peinado et al., 1993) and/or of extensive connections via chemical synapses between cells (Wiggers, 1998). Recently, Wiggers (1998) has reported the existence of dye-coupling between isthmotectal fibers and postsynaptic tectal neurons in salamanders, suggesting a strong connection between cells in these two visual structures. Electron-microscopic studies have indicated that isthmotectal axons terminate on dendrites of tectal neurons, implying that retinotectal and isthmotectal terminals may share the same target within the tectum (Gruberg et al., 1994; Wiggers, 1998). Neuronal connections of this kind may also occur in the tectum of reptiles.

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References

- BENNIS, M., CALAS, A., GEFFARD, M. & GAMRANI, H. (1991). Distribution of GABA immunoreactive systems in the forebrain and midbrain of the chameleon. *Brain Research Bulletin* **26**, 891–898.
- FELIX, D., WANG, S.R., YAN, K. & WANG, Y.T. (1985). The effect of acetylcholine on neurons of the amphibian nucleus isthmi. *Brain Research* **326**, 313–316.
- FELIX, D., WU, G.Y. & WANG, S.R. (1994). GABA as an inhibitory transmitter in the pigeon isthmotectal pathway. *Neuroscience Letters* **169**, 212–214.
- FITE, K.V. & WANG, S.R. (1986). Microiontophoresis and single-unit analysis of cholinergic drugs in the optic tectum of frog. *Brain, Behavior, and Evolution* **28**, 198–206.
- GAO, H.F., WU, G.Y. & WANG, S.R. (1995). Isthmo-tectal neurotransmission in pigeons. *Acta Biophysica Sinica* **11**, 233–236.
- GRUBERG, E.R. (1983). Recent work on the nucleus isthmi and its niche in the visual system. In *Progress in Nonmammalian Brain Research*, Vol. 1, ed. NISTICCO, G. & BOLIS, L., pp. 159–174. Boca Raton, Florida: CRC Press.
- GRUBERG, E.R., HUGHES, T.E. & KARTEN, H.J. (1994). Synaptic interrelationships between the optic tectum and the ipsilateral nucleus isthmi in *Rana pipiens*. *Journal of Comparative Neurology* **339**, 353–364.
- GRUBERG, E.R. & LETTVIN, J.Y. (1980). Anatomy and physiology of a binocular system in the frog *Rana pipiens*. *Brain Research* **192**, 313–325.
- LI, W.C. & WANG, S.R. (1999). Morphology and dye-coupling of cells in the pigeon isthmo-optic nucleus. *Brain, Behavior, and Evolution* **53**, 67–74.
- NORTHMORE, D.P.M. (1991). Visual responses of nucleus isthmi in a teleost fish (*Lepomis macrochirus*). *Vision Research* **31**, 525–535.
- ONN, S.P. & GRACE, A.A. (1994). Dye-coupling between rat striatal neurons recorded *in vivo*: Compartmental organization and modulation by dopamine. *Journal of Neurophysiology* **71**, 1917–1934.
- PEINADO, A., YUSTE, R. & KATZ, L.C. (1993). Extensive dye-coupling between rat neocortical neurons during the period of circuit formation. *Neuron* **10**, 103–114.
- POLLÁK, E., LÁZÁR, G., GÁBRIEL, R. & WANG, S.R. (1999). Localization and source of GABA immunoreactivity in the isthmoc nucleus of the frog *Rana esculenta*. *Brain Research Bulletin* **48**, 343–350.
- RICCIUTI, A.J. & GRUBERG, E.R. (1985). Nucleus isthmi provides most tectal choline acetyltransferase in the frog *Rana pipiens*. *Brain Research* **341**, 399–402.
- SERENÓ, M.I. & ULINSKI, P.S. (1987). Caudal topographic nucleus isthmi and the rostral nontopographic nucleus isthmi in the turtle, *Pseudemys scripta*. *Journal of Comparative Neurology* **261**, 319–346.
- SHERK, H. (1978). Visual-response properties and visual-field topography in the cat's parabigeminal nucleus. *Brain Research* **145**, 375–379.
- SHERK, H. (1979). Connections and visual-field mapping in cat's tecto-parabigeminal circuit. *Journal of Neurophysiology* **42**, 1656–1668.
- VELAZQUEZ, J.L., HAN, D. & CARLEN, P.L. (1997). Neurotransmitter modulation of gap junctional communication in the rat hippocampus. *European Journal of Neuroscience* **9**, 2522–2531.
- WALLACE, M.T., RICCIUTI, A.J. & GRUBERG, E.R. (1990). Nucleus isthmi: Its contribution to tectal acetylcholinesterase and choline acetyltransferase in the frog *Rana pipiens*. *Neuroscience* **35**, 627–636.
- WANG, S.R. (1988). The nucleus isthmus is a visual center: Neuroanatomy and electrophysiology. In *Vision: Structure and Function*, ed. YEW, D.T., SO, K.F. & TSANG, D.S.C., pp. 304–364. Singapore: World Scientific Publishing Co., Pte. Ltd.
- WANG, S.R. & MATSUMOTO, N. (1990). Postsynaptic potentials and morphology of tectal cells responding to electrical stimulation of the bullfrog nucleus isthmi. *Visual Neuroscience* **5**, 479–488.
- WANG, S.R., WANG, Y.C. & FROST, B.J. (1995a). Magnocellular and parvocellular divisions of pigeon nucleus isthmi differentially modulate visual responses in the tectum. *Experimental Brain Research* **104**, 376–384.
- WANG, S.R., WU, G.Y. & FELIX, D. (1995b). Avian Imc-tectal projection is mediated by acetylcholine and glutamate. *NeuroReport* **6**, 757–760.
- WANG, S.R., YAN, K. & WANG, Y.T. (1981). Visual field topography in the frog's nucleus isthmi. *Neuroscience Letters* **23**, 37–41.
- WANG, S.R., YAN, K. & WANG, Y.T. (1982). Nucleus isthmus of toad is secondary visual center. *Scientia Sinica (Ser. B)* **25**, 1172–1178.
- WANG, S.R., YAN, K., WANG, Y.T., JIANG, S.Y. & WANG, X.S. (1983). Neuroanatomy and electrophysiology of the lacertilian nucleus isthmi. *Brain Research* **275**, 355–360.

- WANG, S.R., YAN, K., WANG, Y.T. & WANG, X.S. (1989). Neuronal pathways and morphology of originating cells in the lacertilian midbrain. *Acta Anatomica Sinica* **20**, 159–164.
- WANG, Y.C. & FROST, B.J. (1991). Visual response characteristics of neurons in the nucleus isthmi parvocellularis of pigeons. *Experimental Brain Research* **87**, 624–633.
- WIGGERS, W. (1998). Isthmotectal connections in Plethodontid salamanders. *Journal of Comparative Neurology* **395**, 261–272.
- WIGGERS, W. & ROTH, G. (1991). Anatomy, neurophysiology, and functional aspects of the nucleus isthmi in salamanders of the family *Plethodontidae*. *Journal of Comparative Physiology A* **169**, 165–176.
- WILLIAMS, B., HERNÁNDEZ, N. & VANEGAS, H. (1983). Electrophysiological analysis of the teleostean nucleus isthmi and its relationships with the optic tectum. *Journal of Comparative Physiology A* **152**, 545–554.
- WU, G.Y. & WANG, S.R. (1995). Excitatory and inhibitory transmission from the optic tectum to nucleus isthmi and its vicinity in amphibians. *Brain, Behavior, and Evolution* **46**, 43–49.
- WU, G.Y., WANG, S.R. & FELIX, D. (1994). Effect of acetylcholine and NMDA on neurons of avian tectum and nucleus isthmi. *NeuroReport* **5**, 850–852.
- XIAO, J., WANG, Y. & WANG, S.R. (1999). Glutamatergic, cholinergic, and gabaergic inputs converge on tectal cells in the toad. *Neuroscience* **90**, 1061–1067.
- YAN, K. & WANG, S.R. (1986). Visual responses of neurons in the avian nucleus isthmi. *Neuroscience Letters* **64**, 340–344.
- YAN, K., WANG, Y.T. & WANG, S.R. (1984). A Golgi-Cox study of the lacertilian mesencephalon. *Kexue Tongbao (Science Bulletin)* **29**, 1392–1395.