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Postsynaptic potentials and axonal projections of tegmental neurons responding to electrical stimulation of the toad striatum

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Abstract

The amphibian telencephalic striatum as a major component of the basal ganglia receives multisensory information and projects to the tegmentum and other structures. However, how striatal neurons modulate tegmental activity remains unknown. Here, we show by using intracellular recording and staining in toads that electrical stimulation of the ipsilateral striatum evoked an inhibitory postsynaptic potential (IPSP) in presumably binocular tegmental neurons. Seventy-one neurons were intracellularly stained with Lucifer yellow or horseradish peroxidase. They were located in the anterodorsal tegmental nucleus, anteroventral tegmental nucleus, nucleus profundus mesencephali, and superficial isthmal reticular nucleus, with axons projecting to the tectum, nucleus isthmi, and spinal cord. It appears that the striatum can control visually guided behaviors through the striato-tegmento-spinal pathway and the tegmento-spinal pathway mediated by the tectum and nucleus isthmi.

Keywords: Postsynaptic potentials; Intracellular staining; Striatum; Binocular tegmental neurons; Toads

The telencephalic basal ganglia is highly conserved in vertebrates with respect to neuronal connections, transmitters and functional organization, and plays a key role in integrating multisensory information and transforming perception into action [12,18]. In amphibians, the striatum as a component of the basal ganglia receives visual, auditory, olfactory, and mechanosensory information [2,3]. It is the major telencephalic output to the tegmentum, pretectum, optic tectum and other brain regions [10,11,21]. Lesions of the frog's striatum resulted in severe deficits in visual orientation [17], consisting with the notion that the amphibian striatum is involved in multisensory information processing and visually guided behaviors. Previous studies suggest that prey-catching behaviors in amphibians may involve the disinhibitory striato-pretecto-tectal pathway [6,15].

On the other hand, the tegmentum receives afferents from the striatum and tectum, and projects to the spinal cord [14]. It is most likely that the striato-tegmento-spinal pathway is also essential for controlling visually guided behaviors in amphibians. However, the functional synaptic organization of the striato-tegmental pathway in amphibians is largely unknown. Thus, the present study was undertaken to determine striatal actions on tegmental neurons in toads by using electrical stimulation, intracellular recording and staining techniques.

Experiments were carried out on 38 adult toads (*Bufo bufo gargarizans*) having body length of 6.0–8.5 cm from snout to vent. The animal was immobilized with 0.6–0.8 ml gallamine triethiodide (4%) and covered with wet gauze to facilitate breathing through the skin, and then positioned in a stereotaxic apparatus [22]. The tectum and a forebrain area overlying the striatum on both sides were exposed and the dura mater excised. Procaine (2%) was periodically applied to locally anesthetize the wounds and fixed points. Bipolar tungsten electrodes (20–40 μm tip diameter, 300–400 μm inter-pole distance) were placed in the striatum and on both optic tracts as well. Electrical stimulation was delivered by rectangular pulses of 30–400 μA in intensity and 50–100 μs in duration at 0.1 Hz. Stimulation sites in the basal telencephalon were marked by passing 30 μA current for 10–25 s through the stimulation electrode tip to make electrolytic lesions [19]. Micropipettes (0.5–1.0 μm tip diameter) filled with 3 M potassium acetate and 3% Lucifer yellow (dilithium salt, Sigma Chemical Co., St. Louis, MO) were used for intracellular recording and staining. Tegmental neurons were

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impaled by applying positive pulses (4 nA intensity, 0.3 s duration) and intracellular penetration was indicated by a baseline drop of 30–80 mV. After intracellular recording, the electrode was withdrawn just outside of the impaled cell to record the field potential. Stimulation-evoked postsynaptic potential was obtained by subtracting the field potential from the intracellular potential. Histological procedures for verification of stimulation sites in the striatum by electrolytic lesions and microscopic observation of dye-labeled cells were described in previous studies [19,22]. In some experiments, micropipettes were filled with horseradish peroxidase (4% HRP, Sigma VI, Tris buffer solution, pH 7.4), and HRP was injected into the cells by positive current pulses of 0.5–2 nA in intensity and 500 ms in duration at 0.5 Hz for 2–5 min. After 2–10 h survival, the toad was sacrificed under deep anesthesia and its brain was removed from the skull, fixed in 4% paraformaldehyde for 6–12 h and soaked in 30% sucrose solution in a refrigerator overnight. Frozen sections were cut at 40 μm. Tegmental sections were processed for HRP reactivity [1]. The location and morphology of the stained cells were observed and drawn with a camera lucida attached to a microscope.

One hundred and forty-eight tegmental neurons were intracellularly recorded with resting potentials of negative 30–80 mV and examined for responses to striatal and optic tract stimulations. All these neurons responded to electrical stimulation of the ipsilateral optic tract with an excitatory postsynaptic potential (EPSP, 10%), an EPSP followed by an inhibitory postsynaptic potential (EPSP-IPSP, 85%), or an IPSP alone (5%). The latency of the EPSP was 11.6 ± 3.5 ms (mean ± S.D., $n = 140$) ranging from 6.1 to 22.6 ms. Among the recorded cells, 89.2% (132/148) also responded to electrical stimulation of the contralateral optic tract. Following the contralateral optic tract stimulation, these tegmental neurons produced an EPSP (11%), EPSP–IPSP sequence (79%), or pure IPSP (10%). The EPSP latency ranged from 6.1 to 30.6 ms with an average of 15.5 ± 5.3 ms ($n = 132$) (Fig. 1).

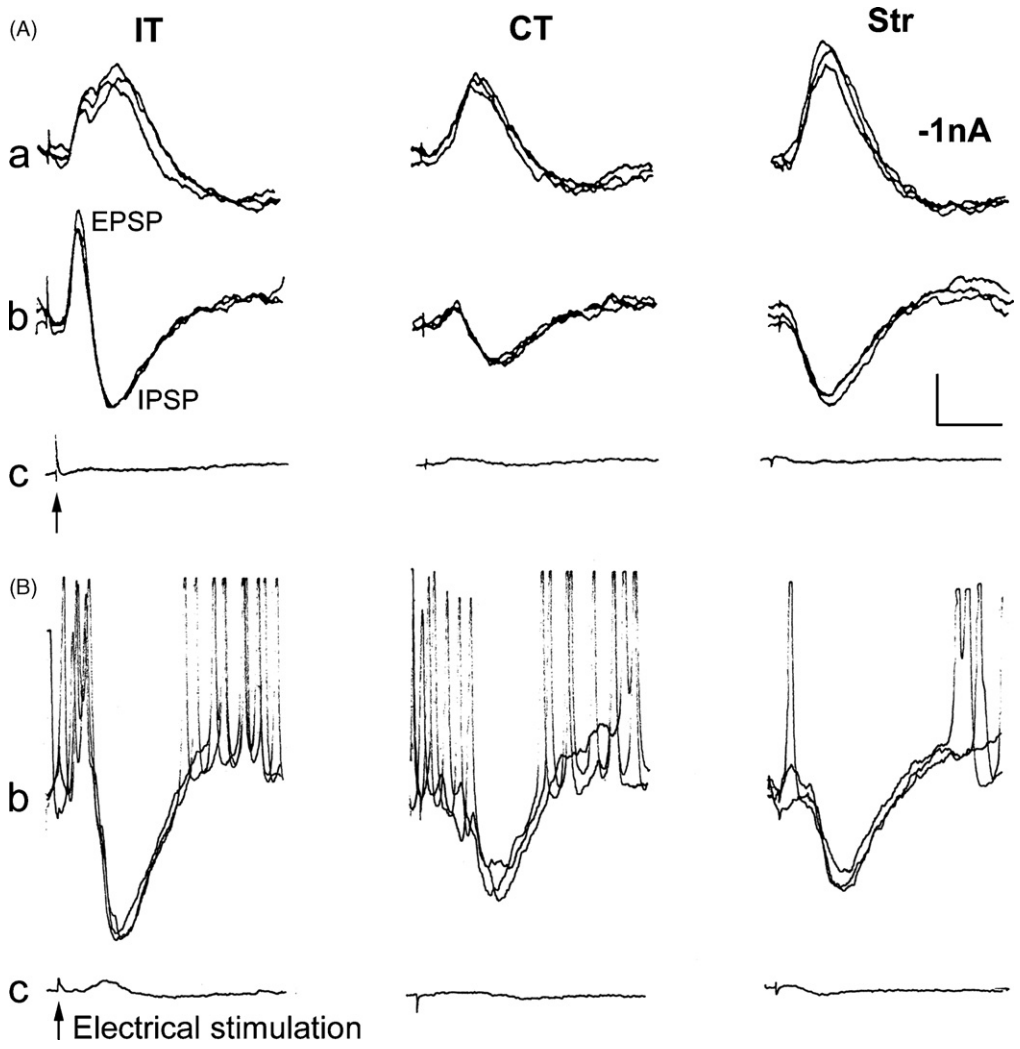

Fig. 1. Postsynaptic responses of two tegmental neurons (A and B) to electrical stimulation of the ipsilateral (IT) and contralateral (CT) optic tracts and of the ipsilateral striatum (Str) and their changes in polarity during hyperpolarizing currents. These cells responded to tract stimulations with excitatory postsynaptic potential (EPSP) followed by inhibitory postsynaptic potential (IPSP) whereas to striatal stimulation with a pure IPSP (b). The IPSP was increased by depolarizing current (not shown) and reversed in polarity by hyperpolarizing current (−1 nA) (A, a). Cell B was spontaneous and its firing rate was increased during EPSP and abolished by IPSP. Horizontal traces beneath postsynaptic responses show field potentials recorded just outside of the impaled cell (c). Three sweeps are superimposed. Arrows point to stimulation artifacts. Scales: 10 mV, 100 ms.

Meanwhile, these tegmental neurons were also examined for intracellular responses to electrical stimulation of the ipsilateral striatum and they all responded with a pure IPSP (Fig. 1A), followed by a rebound excitation in most of the cells. About 25% of the tegmental cells were spontaneously active and their spiking activity was abolished during the IPSP evoked by striatal and tract stimulations (Fig. 1B). The IPSP in tegmental cells evoked by striatal stimulation had an average latency of 19.8 ± 7.0 ms with a range of 9.2–39.8 ms. All these IPSP were graded in amplitude and changed by injections of current. For example, depolarizing current of 1–3 nA increased IPSP in amplitude whereas hyperpolarizing current of similar intensities reduced IPSP and finally reversed its polarity, confirming its IPSP nature.

The amphibian basal ganglia are mainly composed of the striatum and only a small pallidum is found at the caudal basal telencephalon. Thirty-eight stimulation sites in the basal telencephalon were lesioned and all of them were localized in the rostral half of the striatum, which is homologous to the mammalian striatum proper (caudate-putamen) [5,11,18]. Seventy-one neurons were successfully stained with Lucifer yellow or HRP to show their locations and morphological features. Among them, 66 cells were localized in the tegmental nuclei including the anterodorsal nucleus (65%), anteroventral nucleus (4%), nucleus profundus mesencephali (16%), and superficial isthmal reticular nucleus (8%); and 5 others (7%) were unidentified in a particular nucleus (Fig. 2B). They had a variety of morphologies, usually possessing multiangular, piriform or fusiform perikarya and widely branching dendrites. The axons of tegmental neurons were much more frequently labeled and could be traced for a considerable distance by HRP staining compared to Lucifer yellow labeling. Out of the 17 tegmental cells with clearly labeled axons, 10 cells send axons to the deep layers of the ipsilateral tectum and these axons extensively ramify along the projection trajectories (Fig. 2A). None of the tectum-projecting tegmental cells had high spontaneous firing. Four cells project axons to the ipsilateral nucleus isthmi where they ramify widely to contact with isthmic neurons, and three others send axons to the medulla or spinal cord.

The present study found that the majority of the tegmental neurons can be activated by electrical stimulation to either of the optic tracts. This activation is likely transmitted from the tectum to tegmental neurons [14]. The current finding is also supported by our previous studies showing that tegmental cells are activated by both tract stimulations [22]. In fact, some tegmental cells in the anterodorsal nucleus and in the nucleus profundus mesencephali respond to binocular visual stimulations, and others also respond to auditory and tactile stimulations as well [25].

The principal finding of the present study is that the striatum in toads exerts an inhibitory action on tegmental neurons in the anterodorsal nucleus (AD), nucleus profundus mesencephali

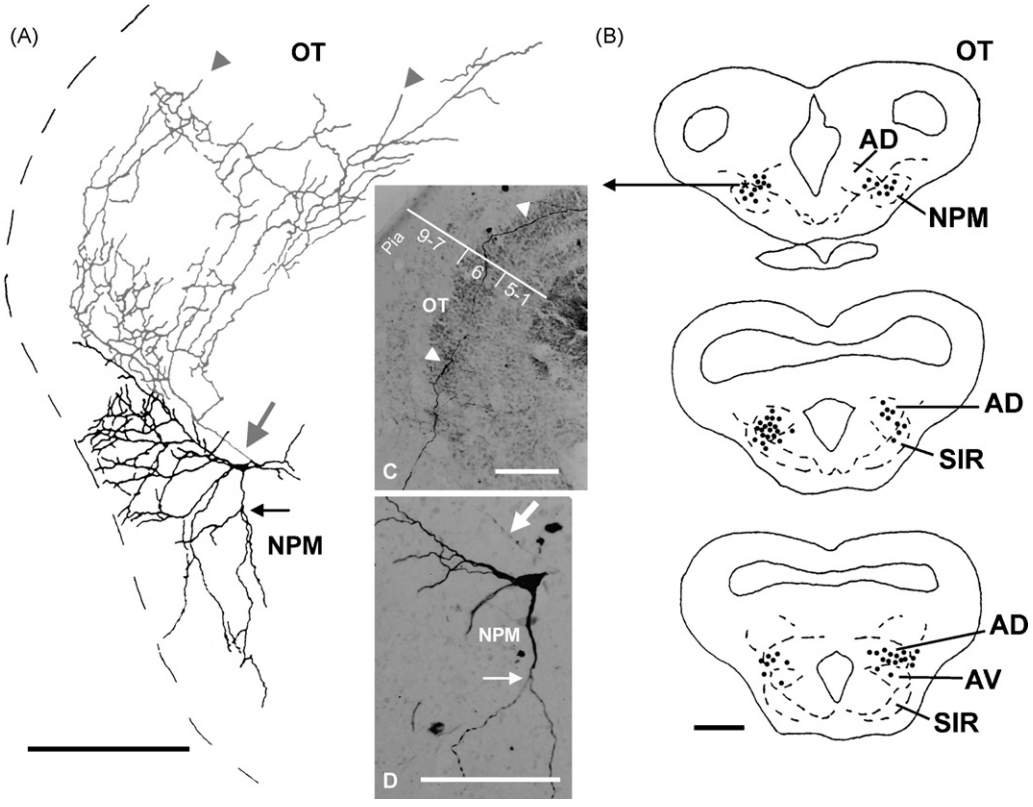

Fig. 2. Topological distribution of the 71 neurons recorded and labeled in the toad's tegmentum and the morphology of an HRP-stained tegmental neuron. These cells (dots) are mainly localized in four tegmental nuclei (AD, NPM, SIR, and AV, see text). Morphological details of an example NPM cell (star with arrow in B) are shown in photomicrographs, demonstrating axonal trajectories in tectum (OT, C), cell body and dendrites in tegmentum (D). Camera lucida drawings (A) show axonal stem (thick arrow) and ramifications (arrowheads) in grey, cell body and dendrites (thin arrow) in black. Anterior-posterior levels of cross-sections of the toad's brain are arranged from top to bottom. Intracellular recording and staining were made in bilateral tegmental. Numerals 1–9 in C represent tectal layers. Scale bars: 500 μm in A and B, 200 μm in C and D.

(NPM), anteroventral nucleus (AV), and superficial isthmal reticular nucleus (SIR). Most of the stained cells are localized within AD and NPM, which are involved in multisensory information processing [25]. The fact that tegmental axons project to the tectum, nucleus isthmi, and spinal cord supports the notion that tegmento-tectal and tegmento-isthmo-tectal pathways can modulate tectal activity [18,19,23,24] and the tegmento-spinal and tecto-tegmento-spinal pathways [14] may control visually guided behaviors. Our results are also consistent with previous studies suggesting that the striato-tegmental pathway is inhibitory and may be mediated by γ-aminobutyric acid (GABA) [4,7,8] with likely involvement of neuropeptides [9]. Together with previous findings, the current results strongly support that these groups of tegmental nuclei as a whole are homologous to the substantia nigra of amniotes. Because the tegmentum reciprocally connects to the optic tectum [14,20] and occupies a key position in the striato-tegmento-tectal [11,21] and tecto-tegmento-spinal [13] pathways, the striato-tegmental pathway could modulate orienting behaviors in amphibians by modulating tectal activity on the one hand, and by controlling the passage of sensory information to premotor centers in the spinal cord on the other.

Amphibians stand on the evolutionary anamniote–amniote transition. The basal ganglia appears to modulate motor functions mainly through its outputs to both cerebral cortex and tectum in amniotes including reptiles, birds, and mammals [18], while this functional modulation may be exerted by its outputs from the striatum to both tectum and tegmentum in anamniotes including amphibians and fish. The inhibitory striato-tegmental pathway we found here may modulate visually guided behaviors in anamniotes at rest [16].

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References

[1] J.C. Adams, Heavy metal intensification of DAB-based HRP reaction product, J. Histochem. Cytochem. 29 (1981) 775.
[2] M. Birkhofer, H. Bleckmann, P. Gorner, Sensory activity in the telencephalon of the clawed toad, *Xenopus laevis*, Eur. J. Morphol. 32 (1994) 262–266.
[3] H. Buxbaum-Conradi, J.P. Ewert, Responses of single neurons in the toad's caudal ventral striatum to moving visual stimuli and test of their efferent projection by extracellular antidromic stimulation/recording techniques, Brain Behav. Evol. 54 (1999) 338–354.
[4] H. Endepols, F. Helmbold, W. Walkowiak, GABAergic projection neurons in the basal ganglia of the green tree frog (*Hyla cinerea*), Brain Res. 1138 (2007) 76–85.
[5] H. Endepols, K. Roden, H. Luksch, U. Dicke, W. Walkowiak, Dorsal striatopallidal system in anurans, J. Comp. Neurol. 468 (2004) 299–310.
[6] J.P. Ewert, H. Buxbaum-Conradi, F. Dreisvogt, M. Glagow, C. Merkel-Harff, A. Rottgen, E. Schurg-Pfeiffer, W.W. Schwippert, Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: perception, attention, motor performance, learning, Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 128 (2001) 417–461.
[7] M.F. Franzoni, P. Morino, The distribution of GABA-like-immunoreactive neurons in the brain of the newt, *Triturus cristatus carnifex*, and the green frog, *Rana esculenta*, Cell Tissue Res. 255 (1989) 155–166.
[8] D.M. Hollis, S.K. Boyd, Distribution of GABA-like immunoreactive cell bodies in the brains of two amphibians, *Rana catesbeiana* and *Xenopus laevis*, Brain Behav. Evol. 65 (2005) 127–142.
[9] G. Lazar, Peptides in frog brain areas processing visual information, Microsc. Res. Technol. 54 (2001) 201–219.
[10] G. Lazar, T. Kozicz, Morphology of neurons and axon terminals associated with descending and ascending pathways of the lateral forebrain bundle in *Rana esculenta*, Cell Tissue Res. 260 (1990) 535–548.
[11] O. Marin, A. Gonzalez, W.J. Smeets, Basal ganglia organization in amphibians: efferent connections of the striatum and the nucleus accumbens, J. Comp. Neurol. 380 (1997) 23–50.
[12] O. Marin, W.J. Smeets, A. Gonzalez, Evolution of the basal ganglia in tetrapods: a new perspective based on recent studies in amphibians, Trends Neurosci. 21 (1998) 487–494.
[13] T. Masino, P. Grobstein, The organization of descending tectofugal pathways underlying orienting in the frog, *Rana pipiens*. II. Evidence for the involvement of a tecto-tegmento-spinal pathway, Exp. Brain Res. 75 (1989) 245–264.
[14] T. Masino, P. Grobstein, Tectal connectivity in the frog *Rana pipiens*: tectotegmental projections and a general analysis of topographic organization, J. Comp. Neurol. 291 (1990) 103–127.
[15] N. Matsumoto, W.W. Schwippert, T.W. Beneke, J.P. Ewert, Forebrain-mediated control of visually guided prey-catching in toads: investigation of striato-pretectal connections with intracellular recording/labeling methods, Behav. Proces. 25 (1991) 27–40.
[16] A. Menard, F. Auclair, C. Bourcier-Lucas, S. Grillner, R. Dubuc, Descending GABAergic projections to the mesencephalic locomotor region in the lamprey *Petromyzon marinus*, J. Comp. Neurol. 501 (2007) 260–273.
[17] P. Patton, P. Grobstein, The effects of telencephalic lesions on visually mediated prey orienting behavior in the leopard frog (*Rana pipiens*). II. The effects of limited lesions to the telencephalon, Brain Behav. Evol. 51 (1998) 144–161.
[18] A. Reiner, L. Medina, C.L. Veenman, Structural and functional evolution of the basal ganglia in vertebrates, Brain Res. Brain Res. Rev. 28 (1998) 235–285.
[19] S.R. Wang, N. Matsumoto, Postsynaptic potentials and morphology of tectal cells responding to electrical stimulation of the bullfrog nucleus isthmi, Vis. Neurosci. 5 (1990) 479–488.
[20] W. Wilczyniski, R.G. Northcutt, Afferents to the optic tectum of the leopard frog: an HRP study, J. Comp. Neurol. 173 (1977) 219–230.
[21] W. Wilczynski, R.G. Northcutt, Connections of the bullfrog striatum: efferent projections, J. Comp. Neurol. 214 (1983) 333–343.
[22] G.Y. Wu, S.R. Wang, Excitatory and inhibitory transmission from the optic tectum to nucleus isthmi and its vicinity in amphibians, Brain Behav. Evol. 46 (1995) 43–49.
[23] G.Y. Wu, S.R. Wang, Extratectal projections to the nucleus isthmi in amphibians, Acta Biophys. Sin. 11 (1995) 401–404.
[24] J. Xiao, Y. Wang, S.R. Wang, Effects of glutamatergic, cholinergic and gabaergic antagonists on tectal cells in toads, Neuroscience 90 (1999) 1061–1067.
[25] K. Yan, S.R. Wang, Y.T. Wang, Visual unit responses in the toad tegmentum, Chin. Sci. Bull. 28 (1983) 1546–1550.