

SPIKING ACTIVITY OF NEURONS IN THE PIGEON NUCLEUS ROTUNDUS IN SLICE PREPARATIONS

Da-Peng Li, Zong-Xiang Tang and Shu-Rong Wang^{CA}

Laboratory for Visual Information Processing, Institute of Biophysics, Chinese Academy of Sciences,
15 Datun Road, Beijing 100101, P.R. China
Corresponding Author; E-mail: wangsr@sun5.ibp.ac.cn

(Accepted March 12, 2004)

SUMMARY

The thalamic nucleus rotundus in birds relays visual information from the mesencephalic tectum to the telencephalic ectostriatum. The present study examined the firing behaviors of rotundal cells in response to depolarizing current injections in pigeon's brain slices. Eighty-five cells examined could be classified into five types according to their firing patterns. Type I cells (58.8%) evoked a spike, bursts or regular spiking depending on current intensity. Type II cells (14.1%) produced a hump-like depolarization that gave rise to a single spike at higher intensity. Type III cells (15.3%) fired a spike or burst only at the onset of current injections. Type IV cells (8.3%) accelerated regular spiking as current intensity increased. Type V cells (3.5%) produced spontaneous spikes that were eliminated by current at higher intensity. The spiking patterns seem to be not correlated to the recording sites. Thirteen neurobiotin-stained cells are multipolar neurons whose morphology is not related to firing patterns. The functional significance of these firing patterns is discussed.

KEY WORDS: brain slice; intracellular recording; morphology; nucleus rotundus; tectofugal pathway

INTRODUCTION

The visual system in nonmammals such as the pigeon is composed of the thalamofugal, tectofugal and accessory optic pathways. The tectofugal pathway in birds, which is thought to be homologous to the colliculo-pulvinar-cortical pathways in mammals, goes from the optic tectum to the ectostriatum via the nucleus rotundus (nRt) (1,18), which receives input from tectal cells of layer 13 (3,11,12,17,22) and projects to the ectostriatum (1,4). This main visual pathway in pigeons is involved in the analysis of intensity, color, motion and patterns of visual stimuli (2,7,8,14). The nRt occupies a key position

between the tectum and the ectostriatum so that revealing the physiological properties of rotundal cells are necessary for the elucidation of visual information processing in the tectofugal pathway.

The nRt is divided into several distinct divisions, which are sensitive to luminance, color, motion or looming stimuli, respectively (32,33). These physiological divisions are supported by subsequent lesion studies (20). The nucleus is also differentially stained for acetylcholinesterase (24). On the other hand, glutamate and γ -aminobutyric acid (GABA) as transmitters and their receptor subtypes seem to be homogeneously distributed throughout nRt (5,16). Excitatory and inhibitory actions of the nucleus of the basal optic root on rotundal cells do not show any regional differences (31). However, little has been known about the firing properties intrinsic to rotundal cells.

Previous studies have shown that there are two types of spiking patterns throughout nRt (15), and that tectal cells projecting to nRt produce bursting and regular spiking patterns in response to depolarizing current injections (23). Some recent studies have indicated that there exist at least five to six firing patterns in the nucleus semilunaris, a component of the tectofugal pathway (29), as well as in the nucleus lentiformis mesencephali and in the nucleus of the basal optic root in pigeons (27,28). It is likely that the pigeon nRt may also possess a similar number of firing patterns in response to intracellular current injections. Therefore, the present study was undertaken by using intracellular recording and staining techniques to reveal the firing patterns intrinsic to rotundal neurons and whether they are division-specific. This knowledge would help in our understanding the mechanisms of visual information processing.

MATERIALS AND METHODS

Forty-four pigeons (*Columba livia*) were used following the guidelines established by the Society for Neuroscience. Each pigeon was anesthetized with ketamine (40mg/100g body weight) and then decapitated. Its brain was immediately removed from the skull, and washed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl, 124; KCl, 5; CaCl₂, 2; MgSO₄, 2; KH₂PO₄, 1.25; NaHCO₃, 26; glucose 10 (10), oxygenated with 95% O₂ plus 5% CO₂. A brain block containing nRt was glued on Vibroslice (Campden Instruments Ltd., UK) and coronally sectioned at 400 μ m thickness. The slices were transferred from a storage container into the recording chamber (BSC-HT, Medical System Corp., USA) perfused with ACSF bubbled with 95% O₂ plus 5% CO₂, and then incubated at 35 \pm 1 for 60 min before being recorded.

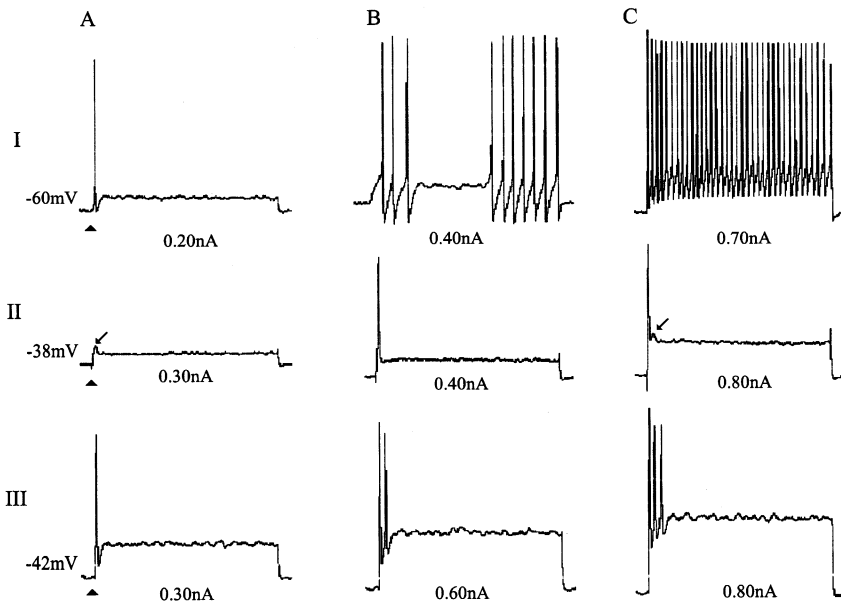
For intracellular recording and staining, a micropipette (0.5-1 μ m tip diameter) filled with either 3 M potassium acetate or 2 M potassium acetate plus 2% neurobiotin (Vector Laboratories Inc., USA) was

advanced into nRt in slices under microscopic control. Rotundal cells were impaled by applying brief positive current pulses (4nA, 0.3s) and impalement was signaled by a sudden d.c. drop. Their firing responses were examined by injecting depolarizing current pulses of 0.05-1.0nA and 300ms on. Intracellular potentials or spikes were amplified (WPI Intra 767, USA), stored on magnetic tapes (RD-135T Data Recorder, TEAC Corp., Japan), and then off-line analyzed with a computer.

In some experiments, neurobiotin was injected with positive current pulses of 1-4nA and 0.4-0.8s on for 2-20 min. After 0.5-2 hrs survival, slices were removed from the recording chamber, fixed in 4% paraformaldehyde and kept in a refrigerator overnight. They were then re-sectioned at 60 μ m thickness. The sections were histologically processed (19,21), and neurobiotin-stained cells photographed at different depths of focus with a microscope. The morphology of rotundal cells was then scanned, reconstructed and measured with computer software Adobe Photoshop.

RESULTS

Intracellular responses of 85 rotundal cells were examined to depolarizing current injections. Their membrane potentials were 53 ± 10 mV (mean \pm S.D) ranging from -80 to -30 mV and recording depths $160 \pm 70\mu$ m throughout nRt. These cells could be classified into five types according to their firing patterns in response to current injections:



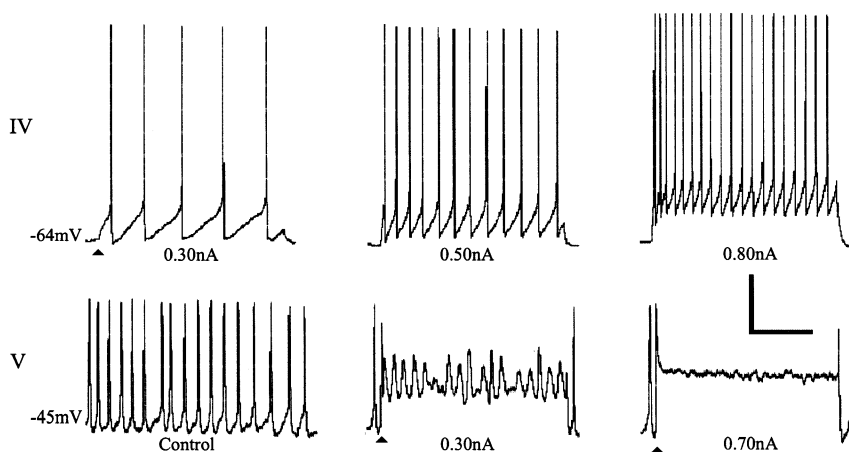


Fig. 1 Rotundal neurons respond to depolarizing current injections in five spiking patterns (I-V). I: Multi-phase spiking cells. II: Single spiking cells. III: Frequency adaptation cells. IV: Regular spiking cells. V: Spiking inactivation cells. Current intensities (nA) are shown in increasing magnitude (A-C) below the recording traces. The resting potentials (mV) of these cells are indicated in the left column. Upward arrowheads point to electrical artifacts at the onset of currents. Oblique arrow points to a hump-like depolarization. Scales: 20 mV, 100 ms.

Type I cells (multi-phase spiking, 58.8%) fired a single spike or burst consisting of 2-9 spikes during threshold current (0.05-0.30nA). An additional group of spikes would occur at the middle or late phase of higher current application (0.20-0.70nA). This intermittent firing was changed to regular firing at a rate of 50-210 spikes/s when current intensity was increased to 0.50-1.00nA (Fig.1-I). These current ranges overlapped because the ranges were different from cell to cell. Type II cells (single spiking, 14.1%) responded to threshold current (0.20-0.40nA) with a hump-like depolarization, which would become a single spike at higher intensity (0.30-0.70nA). Further increase in intensity (0.50-1.00nA) resulted in a single spike followed by a hump-like depolarization (Fig.1-II). Type III cells (frequency adaptation, 15.3%) fired a single spike during threshold current (0.10-0.40nA). Higher intensity (0.30-1.00nA) produced a burst containing 2-6 spikes at 50-120 spikes/s, followed by an adaptation period without spiking activity (Fig.1-III). Type IV cells (regular spiking, 8.3%) responded with regular spikes (10-46 spikes/s) to threshold current (0.10-0.30nA). Further increase in intensity (0.20-1.00nA) led to an increase in firing rate to 30-140 spikes/s (Fig.1-IV). Type V cells (spiking inactivation, 3.5%) spontaneously fired at 50-120 spikes/s, and these spikes were reduced in amplitude to small ones during threshold current (0.10-0.30nA). They were further reduced in amplitude by increasing current intensity and completely abolished when intensity was increased to 0.30-0.70nA (Fig.1-V).

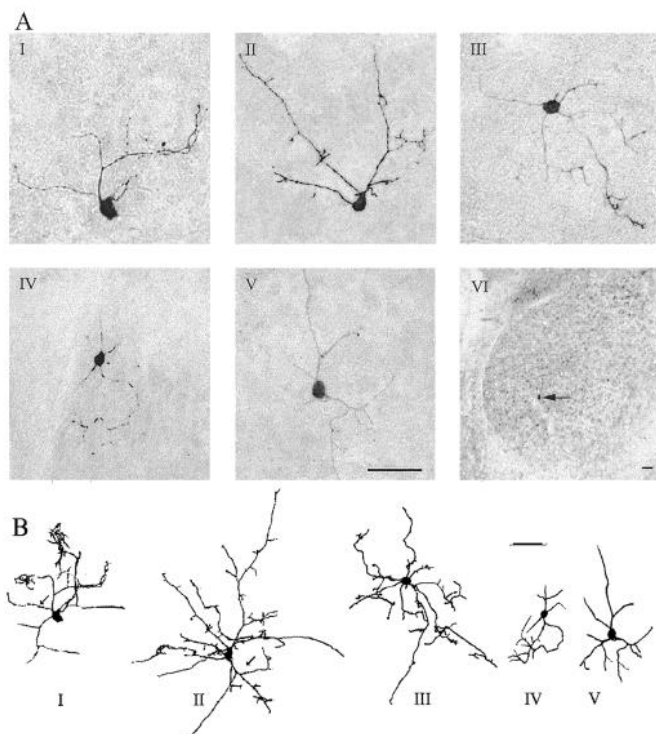


Fig. 2 Photomicrographs (A, I-V) and computer-aided reconstruction drawings (B, I-V) of electrophysiologically identified rotundal neurons. Drawings in (B) are reconstructed from several photographs of a cell taken at different depths of focus. Sections I-V in (A) correspond to those with the same numerals in (B). Arrows in (B-I, II) indicate dendritic varicosities. Photo at the low-right corner (a) is a cross section (counterstained with cresyl violet) at the nRt level shows the position of the type I neuron, whose photo is at the top-left, in the ventral nRt (horizontal arrow). Scale bars: 100 μm .

The recording sites of 85 cells were distributed throughout nRt, showing no apparent correlation between the localization and physiological types of nRt cells examined. The morphological features of 13 rotundal cells (6 type I, 3 type II, 1 type III, 2 type IV, and 1 type V cells) were demonstrated with neurobiotin staining. All these were multipolar, radiating 4-8 primary dendrites that usually gave rise to secondary dendrites with branches, but an axon was seldom observed. Numerous dendrites bore varicosities, which are local enlargements in dendrites giving an appearance of beads. Fig. 2 shows the photomicrographs and morphological reconstructions of some rotundal cells, which represent each of the spiking patterns found in the present study.

DISCUSSION

The firing patterns of a neuron in response to depolarizing current injections are intrinsic to the neuron, because it is actually deprived of all external inputs from other neurons *in vitro*. The present study finds three more types (III-V) of spiking patterns in nRt than the previous study (15). Type I cells in both studies are characterized by multi-phase spiking. Though type II cells in the present study also produce a hump-like depolarization as in the previous study, but they fire a large single spike when higher intensity current is applied. All these discrepancies might be due to three facts: (a) slice incubation temperature is higher here than that used before (35 vs. 30 °C); (b) type II in the previous study may result from lower slice vitality though it has also been reported in other species (25); and (c) a sample here is twice that reported before (85 vs. 41 cells). Frequency adaptation (type III) and regular spiking (type IV) cells are also found in the accessory optic nuclei (27,28), as well as in tectal cells (10,23,25). Spiking inactivation (type V) cells are also found in the pigeon nucleus lentiformis mesencephali (28). Generally speaking, various nuclei in the pigeon visual system produce similar spiking patterns in response to current injections. Most of these spiking patterns also occur in some other species (9,25). It is likely that several firing patterns might be fundamental and could be used for coding various aspects of visual information depending on the afferent input and synaptic connections of retinal neurons.

Some fundamental firing patterns could be combined into a complex pattern. For example, multi-phase spiking cells (type I) may be a combination of late-spiking, fast-spiking and frequency-adaptation cells observed in the rat superior colliculus (25). Their spiking patterns could change from one to another depending on the intensity of depolarizing currents. The firing patterns intrinsic to neurons may be indicative of their physiological functions. For example, single spiking cells (type II) is recently reported in the frog tectum and suggested to stem from ganglionic neurons, some of which probably respond to diffuse light stimulation (9). Rapid cessation of firing activity might result from inactivation of sodium-channels (25). Frequency adaptation cells (type III) fired a single or burst of spikes at the onset of current application depending on intensity, suggesting that they might respond physiologically to the onset of stimulus motion or light stimulation. Regular spiking mode (type IV) is most frequently observed on *in vivo* and *in vitro* preparations because it can perform a linear summation of excitatory responses (26), signaling the strength of stimulation. The proportion of spiking inactivation cells (type V) in nRt is quite small, as in the pigeon nucleus lentiformis mesencephali (28).

Though nRt in birds contains several anatomical (3,11,12) and physiological (6,20,32,33) divisions, the present study does not show a division-specific distribution of the spiking patterns in nRt. This apparently homogeneous distribution might be a bias due to a small sample of cells examined. However, it is more likely that such firing patterns may be fundamental to a variety of neurons and their interactions with visual inputs to different divisions result in the physiological divisions found in nRt. In fact, any nRt neuron in vivo works in a neuronal network, which could be activated by visual inputs from the tectum (3,11,12,17,22), the SP/IPS complex (3,30), the nucleus semilunaris (13), and/or the nucleus of the basal optic root (31). To reveal the physiological interactions between intrinsic firing patterns and external visual inputs, further studies would be needed by combining intracellular recording and visual stimulation in animals.

This work was supported by the National Natural Science Foundation of China and by the Brain-Mind Project of Chinese Academy of Sciences.

REFERENCES

1. Benowitz LI, Karten HJ. Organization of the tectofugal visual pathway in the pigeon: a retrograde transport study. *J Comp Neurol* 1976; 167: 503-520.
2. Bessette BB, Hodos W. Intensity, colour, and pattern discrimination deficits after lesions of the core and belt regions of the ectostriatum. *Vis Neurosci* 1989; 2: 27-34.
3. Deng C, Rogers LJ. Organization of the tectorotundal and SP/IPS-rotundal projection in the chick. *J Comp Neurol* 1998; 394: 171-185.
4. Engelage J, Bischof HJ. The organization of the tectofugal pathway in birds: A comparative review. In: Zeigler HP, Bischof HJ, editor. *Vision, Brain, and Behavior in Birds*. MIT Press; 1993. p137-158.
5. Gao HF, Wu GY, Frost BJ, Wang SR. Excitatory and inhibitory neurotransmitters in the nucleus rotundus of pigeons. *Vis Neurosci* 1995; 12: 819-825.
6. Granda AM, Yazulla S. The spectral sensitivity of single units in the nucleus rotundus of pigeon, *Columba livia*. *J Gen Physiol* 1971; 57: 363-384.
7. Gu Y, Wang Y, Wang SR. Regional variation in receptive field properties of tectal cells in pigeons. *Brain Behav Evol* 2000; 55: 221-228.
8. Gu Y, Wang Y, Zhang T, Wang SR. Stimulus size selectivity and receptive field organization of ectostriatal neurons in the pigeon. *J Comp Physiol A* 2002; 188: 173-178.
9. Gutmaniene N, Svirskiene N, Svirskis G. Firing properties of frog tectal neurons in vitro. *Brain Res* 2003; 981: 213-216.
10. Hardy O, Audinat E, Jassik-Gerschenfeld D. Electrophysiological properties of neurons recorded

- intracellularly in slices of the pigeon optic tectum. *Neuroscience* 1987; 23: 305-318.
11. Hellmann B, Güntürkün O. Visual-field-specific heterogeneity within the tecto-rotundal projection of the pigeon. *Eur J Neurosci* 1999; 11: 2635-2650.
 12. Hellmann B, Güntürkün O. Structural organization of parallel information processing within the tectofugal visual system of the pigeon. *J Comp Neurol* 2001; 429: 94-112.
 13. Hellmann B, Manns M, Güntürkün O. Nucleus isthmi, pars semilunaris as a key component of the tectofugal visual system in pigeons. *J Comp Neurol* 2001; 436: 153-166.
 14. Hodos W, Karten HJ. Brightness and pattern discrimination deficits in the pigeon after lesions of nucleus rotundus. *Exp Brain Res* 1966; 2: 151-167.
 15. Hu J, Wang SR. Firing patterns and morphological features of neurons in the pigeon nucleus rotundus. *Brain Behav Evol* 2001; 57: 343-348.
 16. Huang LH, Li JL, Wang SR. Glutamatergic neurotransmission from the optic tectum to the contralateral nucleus rotundus in pigeons. *Brain Behav Evol* 1998; 52: 55-60.
 17. Karten HJ, Cox K, Mpodozis J. Two distinct populations of tectal neurons have unique connections within the retinotectorotundal pathway of the pigeon (*Columba livia*). *J Comp Neurol* 1997; 387: 449-465.
 18. Karten HJ, Hodos W. Telencephalic projections of the nucleus rotundus in the pigeon (*Columba livia*). *J Comp Neurol* 1970;140: 35-52.
 19. Kita H, Armstrong W. A biotin-containing compound N-(2-aminoethyl) biotinamide for intracellular labeling and neuronal tracing studies: comparison with biocytin. *J Neurosci Meth* 1991; 37: 141-150.
 20. Laverghetta AV, Shimizu T. Visual discrimination in the pigeon (*Columba livia*): Effects of selective lesions of the nucleus rotundus. *NeuroReport* 1999; 10: 981-985.
 21. Li WC, Perrins R, Soffe SR, Yoshida M, Walford A, Roberts A. Defining classes of spinal interneuron and their axonal projections in hatchling *Xenopus laevis* tadpoles. *J Comp Neurol* 2001; 441: 248-265.
 22. Luksch H, Cox K, Karten HJ. Bottlebrush dendritic endings and large dendritic fields: motion-detecting neurons in the tectofugal pathway. *J Comp Neurol* 1998; 396: 399-414.
 23. Luksch H, Karten HJ, Kleinfeld D, Wessel R. Chattering and differential signal processing in identified motion-sensitive neurons of parallel visual pathways in the chick tectum. *J Neurosci* 2001; 21: 6440-6446.
 24. Martinez-de-la-Torre M, Martinez S, Puelles L. Acetylcholinesterase histochemical differential staining of subdivisions within the nucleus rotundus in the chick. *Anat Embryol* 1990; 181: 129-135.
 25. Saito Y, Isa T. Electrophysiological and morphological properties of neurons in the rat superior colliculus. I. Neurons in the intermediate layer. *J Neurophysiol* 1999; 82: 754-767.
 26. Sherman SM. Tonic and burst firing: dual modes of thalamocortical relay. *Trends Neurosci* 2001; 24: 122-126.
 27. Tang ZX, Wang SR. Discharge patterns evoked by depolarizing current injection in basal optic nucleus neurons of the pigeon. *Brain Res Bull* 2002; 58: 371-376.
 28. Tang ZX, Wang SR. Intracellular recording and staining of neurons in the pigeon nucleus lentiformis mesencephali. *Brain Behav Evol* 2002; 60: 52-58.
 29. Tang ZX, Wang SR. Firing properties and dye coupling of neurons in the pigeon nucleus semilunaris. *Brain Behav Evol* 2003; 62: 175-181.

30. Tömböl T, Németh A, Sebestény T, Alpár A. Electron microscopic data on the neurons of nuclei subpretectalis and posterior-ventralis thalami. A combined immunohistochemical study. *Anat Embryol* 1999; 199: 169-183.
31. Wang Y, Gu Y, Wang SR. Modulatory effects of the nucleus of the basal optic root on rotundal neurons in pigeons. *Brain Behav Evol* 2000; 56: 287-292.
32. Wang YC, Frost BJ. Time to collision is signaled by neurons in the nucleus rotundus of pigeon. *Nature* 1992; 356: 236-238.
33. Wang YC, Jiang SY, Frost BJ. Visual processing in pigeon nucleus rotundus: Luminance, color, motion, and looming subdivisions. *Vis Neurosci* 1993; 10: 21-30.