

Firing Properties and Dye Coupling of Neurons in the Pigeon Nucleus semilunaris

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Key Words

Birds · Brain slice · Dye coupling · Tectofugal pathway · Vision

Abstract

Our previous study indicated that the nucleus semilunaris in birds is a visual center. The present study using pigeon brain slices shows that 84 semilunar cells examined could be grouped into five types according to responses to depolarizing current injections. Type I cells (early bursting, 44%) fire a single burst followed by regular spiking. Type II cells (regular spiking, 13%) regularly produce spikes, the rates of which are enhanced as currents are increased. Type III cells (bursting, 17%) discharge a series of bursts each consisting of 2–4 spikes. Type IV cells (dual spiking, 15%) evoke both spikes and spikelets. Type V cells (inhibition-following, 11%) are characterized by regular spiking followed by an inhibitory period after current cessation. Morphologically, semilunar neurons have piriform, round, or fusiform somata of 12–23 μm in diameter, which give rise to 2–4 primary dendrites with sparse branches. Dual spiking activity is invariably correlated with dye coupling, and bursting cells have a tendency to be fusiform in shape. Other

types of semilunar cells do not show a correlation between their firing patterns and morphological features.

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Introduction

The optic tectum in birds is the principal destination of optic fibers from the contralateral retina. In the subtectal region are the isthmic nuclei, which are composed of the nucleus isthmi pars magnocellularis (Imc), the nucleus isthmi pars parvocellularis (Ipc), and the nucleus semilunaris (Slu). Both Ipc and Slu are connected reciprocally and topographically with the ipsilateral tectum [Hunt and Künzle, 1976; Güntürkün and Remy, 1990; Hellmann et al., 2001]; Imc possibly receives tectal input [Hunt and Künzle, 1976] and projects back ipsilaterally [Wang and Wang, 1990; Tömböl and Németh, 1998]. The isthmo-optic nucleus is not included in the isthmic nuclei because it receives input from the tectum but projects centrifugally to the retina [Uchiyama, 1989].

Extensive studies have shown that the isthmic nuclei in non-mammals including fishes, amphibians, reptiles and birds are all visual centers [see Wang, 2003]. In birds, Imc

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and Ipc exert excitatory and inhibitory actions on tectal cells [Felix et al., 1994; Wang et al., 1995a, b], and thereby modulate the excitatory center and the inhibitory surround of the receptive field of tectal cells, respectively [Wang et al., 2000; Wang, 2003]. Although Slu occupies an important position in the tectofugal system of pigeons [Hellmann et al., 2001], our knowledge about the physiology and the morphology of Slu neurons is still scarce. An early Golgi study briefly described the morphology of semilunar cells [Güntürkün, 1987]. We have recently shown that Slu is a visual center in the pigeon midbrain [Yang et al., 2002]. To further reveal the physiological and morphological features of Slu cells in pigeons, the present study was carried out by using intracellular recording and staining techniques in brain slice preparations.

Materials and Methods

Thirty-two adult pigeons (*Columba livia*) were used following the policy on the use of animals established by the Society for Neuroscience. The experimental procedures were described previously in detail [Tang and Wang, 2002a,b]. Briefly, each pigeon was anesthetized with ketamine hydrochloride and then decapitated. The brain was immediately removed and washed in ice-cold Krebs-Ringer solution that contained (in mM) NaCl, 124; KCl, 5; CaCl₂, 2; MgSO₄, 2; KH₂PO₄, 1.25; NaHCO₃, 26; glucose 10 [Hardy et al., 1987] and bubbled with 95% O₂ plus 5% CO₂. A block containing Slu was glued onto the stage of a Vibroslice (Campden Instruments Ltd., Loughborough, Leics., UK), and coronally sectioned at 400 μm. Slices were transferred from a storage container to the recording chamber (BSC-HT, Medical System Corp., Greenvale, N.Y., USA) perfused with Krebs-Ringer solution bubbled with 95% O₂ plus 5% CO₂ at a rate of 2 ml/min. They were incubated at 35°C for 60 min before recording.

A micropipette (0.5–1 μm tip diameter) filled with either 3M potassium acetate or 3% Lucifer yellow (dilithium salt, Sigma Chemical Co., St. Louis, Mo., USA) plus 0.1 M LiCl [Onn and Grace, 1994] was advanced into Slu under microscopic control. Cells were impaled by applying positive pulses (4 nA, 0.3 s duration) and intracellular insertion was signaled by a baseline drop of 30–80 mV. The firing behaviors of Slu cells were examined by injecting depolarizing pulses of 0.05–1.00 nA in intensity and 0.2–0.4 s in duration. Intracellular responses were amplified, displayed on a digital oscilloscope (VC-7104, Hitachi Denshi Ltd., Tokyo), stored on magnetic tapes (RD-135T Data Recorder, TEAC Corp., Tokyo) and then off-line analyzed.

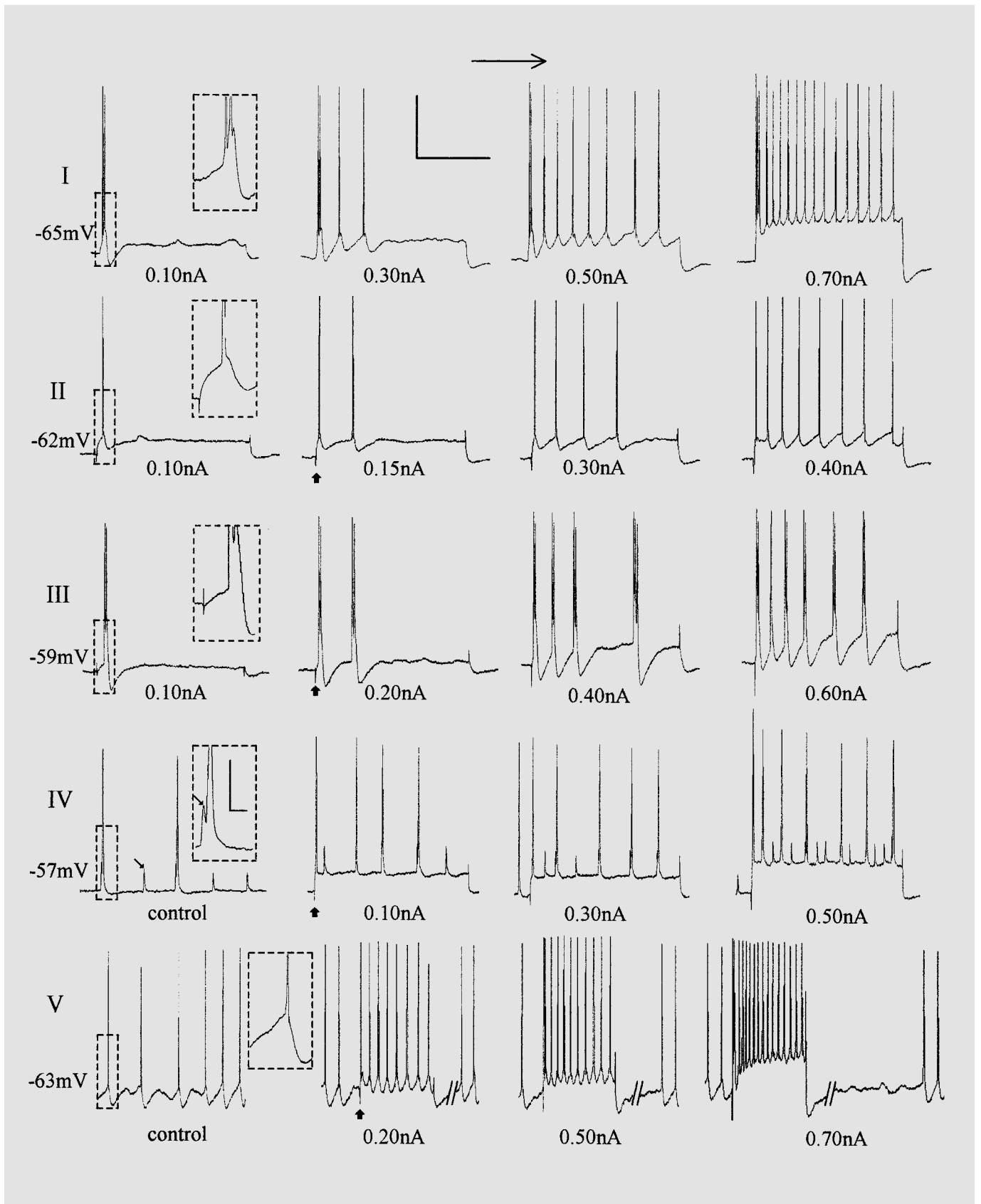
Dye was injected by passing negative pulses (2–4 nA, 1–2 Hz, 2–20 min) with one injection per slice in most cases. After 0.5–2 h survival, the slices were fixed in 4% paraformaldehyde and kept in a refrigerator overnight. They were rinsed with physiological saline and then placed in 100% dimethylsulfoxide (DMSO) for 20 min [Grace and Llinàs, 1985]. The dye-marked cells were photographed at various depths of focus with a fluorescence microscope (Nikon Microphot FXA, BV filter). The morphology of cells was scanned with a scanner (StudioStar, AGFA, Hong Kong) into a computer and reconstructed with software tools of Adobe Photoshop 5.0.

Results

Eighty-four Slu cells were examined for their intracellular responses to depolarizing current injections, 32 of which were stained to show their morphological features. The recording depths ranged from 30 to 290 μm, and resting potentials were between –30 and –80 mV with an average of -52 ± 10 mV (mean \pm S.D, $n = 84$) (fig. 1).

These cells could be grouped into five types according to their responses to depolarizing currents. Type I included 37 early bursting cells (44%), which fired a burst consisting of 2–3 spikes at threshold intensities (0.05–0.30 nA). An increase in current intensities up to 0.90 nA elicited a burst of 2–3 spikes followed by regular spiking at frequencies of 10–70 spikes/s depending on current intensities. Type II included 11 regular spiking cells (13%), which fired a single spike at threshold intensities (0.10–0.20 nA) and regular spiking at rates of 7–54 spikes/s at higher intensities ranging from 0.20 to 0.80 nA. Type III consisted of 14 bursting cells (17%) which evoked a burst of 2–3 spikes at threshold intensities (0.10–0.20 nA) and a series of bursts at frequencies of 7–27 bursts/s with 2–4 spikes in each burst at higher intensities (0.20–0.80 nA). Type IV included 13 dual spiking cells (15%) that fired both spikes and spikelets. The average amplitude of spikes was 41 mV and that of spikelets was 8 mV. Among these, 11 cells increased rates of both spikes and spikelets and two others increased rates of spikes but decreased that of spikelets as current intensities were increased. Nine inhibition-following cells (11%) of type V had an average spontaneous rate of 11 spikes/s and fired 15–32 spikes/s during currents of 0.10–0.30 nA, followed by an inhibition period of 0.20–0.40 s after current cessation. Further increases in intensities produced higher firing

Fig. 1. Five firing patterns of pigeon's semilunar neurons in response to depolarizing current injections which were respectively recorded from early bursting (I), regular spiking (II), bursting (III), dual spiking (IV), and inhibition-following (V) cells. Current intensities (nA) are shown in increasing magnitude (horizontal arrow) below recording traces. The resting potential (mV) of each cell is indicated on the left. Upward arrows point to electrical artifacts at the onset of current injections. Oblique arrows (IV) point to spikelets. In some cases, spikelets are in the form of prepotentials shown in inset (IV). Insets are an enlarged part of the first spikes framed by dashed rectangles. Scales: 150 ms in I–IV and 300 ms in V, and 20 mV. In insets, 10 ms in I–IV and 20 ms in V, and 10 mV. Interrupted interval represents 300 ms.



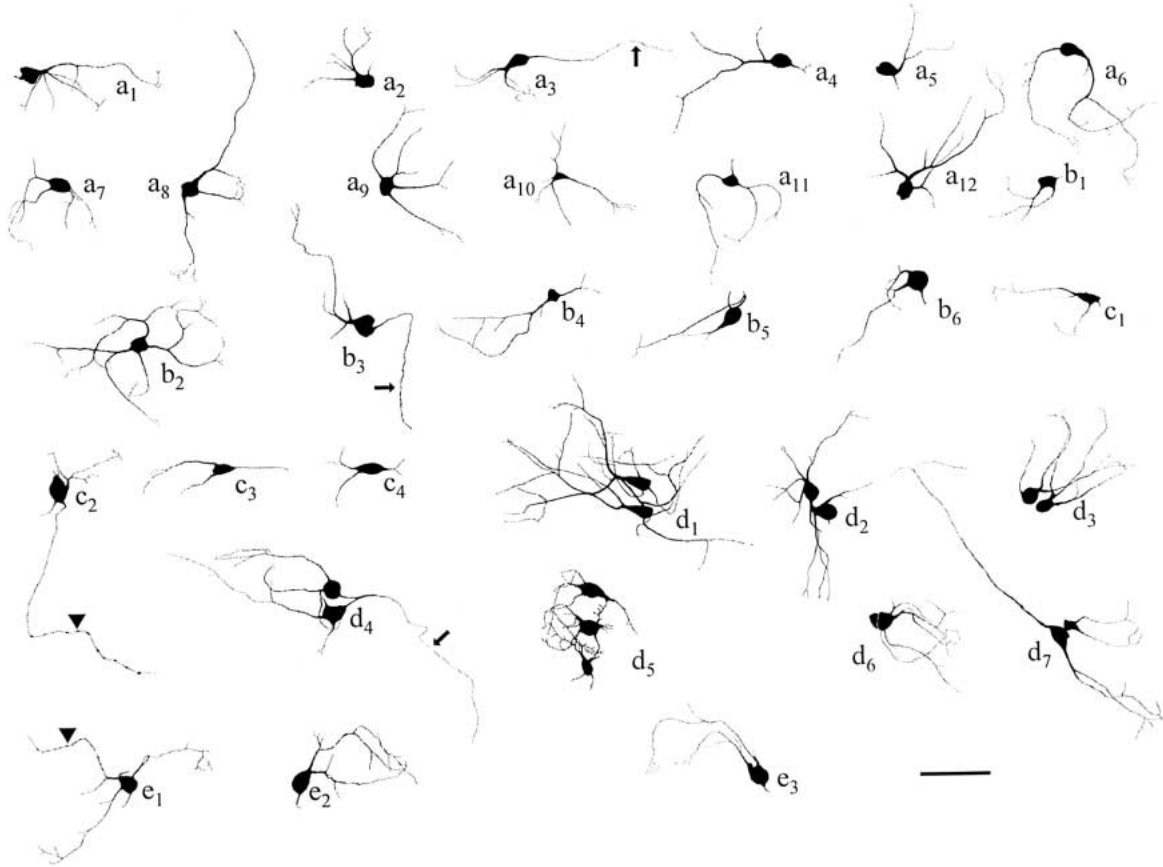
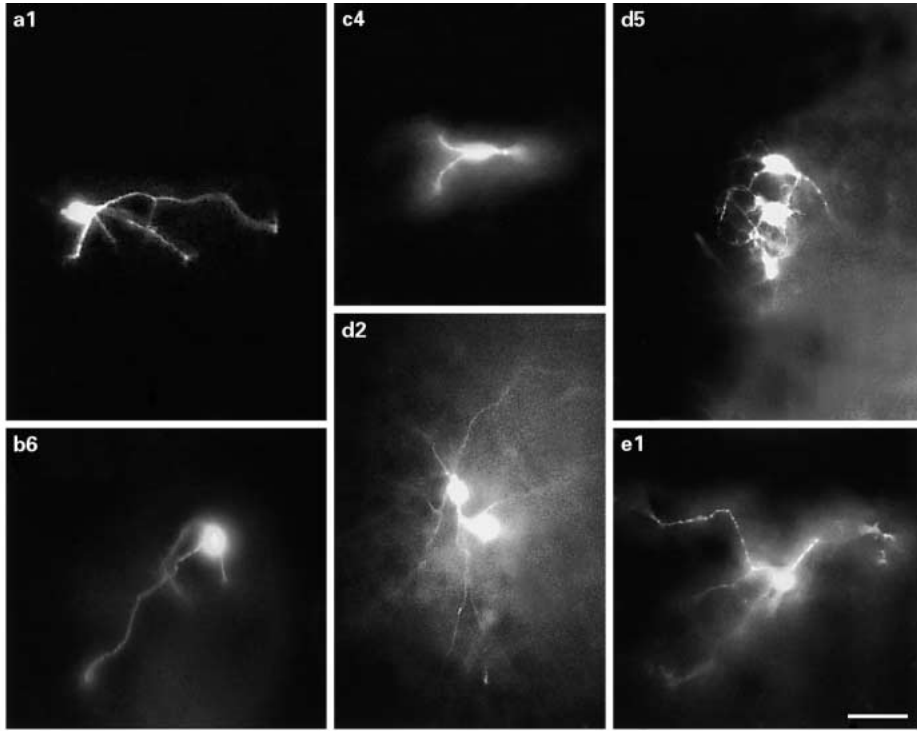


Table 1. Relationship between current intensities and firing rates in five types of semilunar neurons

Type	Current (nA)	0.10	0.30	0.50	0.70
I	spikes/s	5 ± 3	15 ± 7	29 ± 14	42 ± 14 (n = 37)
II	spikes/s	3 ± 2	15 ± 8	31 ± 10	47 ± 7 (n = 11)
III	bursts/s	2 ± 1	8 ± 3	13 ± 5	21 ± 4 (n = 14)
IV	spikes/s	9 ± 6	16 ± 6	28 ± 8	35 ± 7 (n = 11)
	spikelets/s	8 ± 4	19 ± 10	30 ± 10	38 ± 12 (n = 11)
V	spikes/s	15 ± 2	31 ± 8	45 ± 9	58 ± 12 (n = 9)
	inhibition, ms	97 ± 38	230 ± 105	403 ± 126	620 ± 181 (n = 9)

Inhibition represents an inhibition period (ms) following current cessation.

rates and a longer-lasting inhibition period. The relationship between current intensities and firing rates in each type of Slu cells is summarized in table 1.

Thirty-two intracellularly recorded cells were stained with dye. Early bursting cells (fig. 2a₁₋₁₂) had piriform and round somata of $18.3 \pm 2.5 \mu\text{m}$. They gave rise to 2–4 primary dendrites bearing sparse branches. An axon in cell a₃ originated from soma and traveled medially. Regular spiking cells (b₁₋₆) had a similar shape and size ($18.5 \pm 3.2 \mu\text{m}$) to early bursting cells. An axon in cell b₃ originated from the soma and went dorsomedially and then ventrally. Bursting cells (c₁₋₄) possessed fusiform somata of $19.3 \pm 1.6 \mu\text{m}$ in size. Their somata issued 2–3 dendrites with few branches. An axon in cell c₂ bore some varicosities, running ventrally and then ventromedially. The prominent feature of dual spiking cells (d₁₋₇) was multiple labeling consisting of 2–3 cells in a cluster. Their somata were piriform, fusiform and round in shape, and 5–26 μm spaced (d₁₋₅) or apposed together (d_{6,7}). The soma size of the spaced cells was $19.0 \pm 2.4 \mu\text{m}$ (n = 11) and that of the apposed twin cells was $17.0 \pm 1.4 \mu\text{m}$ (n = 4). An axon d₄ was observed traveling ventromedially. These cells might make dendro-dendritic (d_{1-3, 5}), axo-axonal (d₄), or so-

mato-somatic (d_{6,7}) couplings. The somata of inhibition-following cells (e₁₋₃) were piriform and had an average size of 20 μm ranging from 19 to 21 μm . They issued 2–4 primary dendrites with a few branches which occasionally bore some varicosities. All these labeled cells were distributed throughout the nucleus, showing no observable correlation between their locations and physiological types.

Discussion

Our previous study indicated that Slu is a visual center in the pigeon midbrain [Yang et al., 2002]. The present study reveals five firing types of semilunar cells in response to depolarizing current injections.

Early bursting cells predominate within Slu. This firing pattern has been found in the visual system of various species [Gray and McCormick, 1996; Mancilla et al., 1998; Saito and Isa, 1999; Tang and Wang, 2002a], and might underlie in part the onset responses, i.e., transient responses of visual cells to onset of stimuli. Regular spiking and bursting cells also exist in the nucleus lentiformis mesencephali [Tang and Wang, 2002b], in the nucleus of the basal optic root [Tang and Wang, 2002a], and in the tectum [Luksch et al., 2001] in birds. Regular spiking is most frequently observed in excitatory responses because it can linearly sum to reflect stimulus strengths [Sherman, 2001]. Neurons in the cortex [Gray and McCormick, 1996; Mancilla et al., 1998] and tectum [Hardy et al., 1987; Luksch et al., 2001] might respond in a bursting mode, which could reliably signal that something has changed in the environment [Sherman, 2001]. In fact, some neurons in the pigeon tectum fire a series of bursts in response to a small moving target, probably due to the discontinuous structures of the receptive fields [Troje and Frost, 1998].

Fig. 2. Photomicrographs of some dye-labeled semilunar neurons (top) and computer-aided drawings of 32 physiologically identified semilunar neurons (bottom). Morphological drawings of cells in photographs are shown with corresponding labels. The cellular morphologies correspond to early bursting (a₁₋₁₂), regular spiking (b₁₋₆), bursting (c₁₋₄), dual spiking (d₁₋₇), and inhibition-following (e₁₋₃) cells, respectively. Note the correlation between dual spiking activity and multiple labelings. Triangles point to varicosities in an axon (c₂) and dendrite (e₁). Arrows point to axons (a₃, b₃, d₄). Scale bars: 80 μm in drawings, 50 μm in photographs.

In dual spiking cells, spikelets might represent attenuated action potentials from cells coupled electrotonically via gap junctions [Hughes et al., 2002], but not fast excitatory postsynaptic potentials, dendritic spikes, or axon hillock spikes [Grace and Bunney, 1983]. The present study shows that in most cases both spikes and spikelets increase firing rates concurrently, and in other cases the spike rate increases, whereas that of spikelets decreases. These could imply the existence of two mechanisms for modulating gap communication, positive and negative, depending on intracellular pH, voltage, transmitters and second messengers [Perez Velazquez and Carlen, 2000]. Gap communication has been suggested to play important roles in synchronization of neuronal assemblies [Perez Velazquez and Carlen, 2000], in the generation and stabilization of bursting behavior [Skinner et al., 1999] and in fast axon-axonal communication [Schmitz et al., 2001]. Inhibition-following cells produce tonic responses to current injection and inhibitory responses to current cessation. The inhibition duration is prolonged as current intensity is increased. This might be adaptation or fatigue following excitatory responses.

Semilunar neurons stained in the present study are morphologically similar to those obtained by Golgi impregnation [Güntürkün, 1987]. However, some neurons bear dendritic varicosities, and this does not favor the finding that all semilunar neurons bear dendritic spines [Güntürkün, 1987]. This discrepancy might be due to intracellular vs. Golgi staining. The present study finds the existence of dye couplings between semilunar cells in

15% of the cases. They are invariably correlated with dual amplitude spikes, of which spikelets appear to be characteristic of electrotonic coupling via gap junctions [Grace and Bunney, 1983; Hughes et al., 2002]. Bursting cells tend to be fusiform in shape. Other types of cells do not show any correlation between physiology and morphology.

In view of the fact that Slu and Ipc are reciprocally connected with tectum in a topographical fashion [Hunt and Künzle, 1976; Güntürkün and Remy, 1990; Hellmann et al., 2001], this isthmic complex might be homologous to the nucleus isthmi in amphibians, Imc in reptiles and the parabigeminal nucleus in mammals [see Wang, 2003]. Anatomical and histochemical studies recently found that both Slu and Ipc in chicks receive inhibitory input from Imc, and they might participate in tectal motion detection [Wang Y, Major DE, Karten HJ, personal communication]. On the other hand, electrophysiological studies reported that Imc and Ipc in pigeons respectively modulate the excitatory center and the inhibitory surround of the receptive field of tectal cells [Wang et al., 2000]. Further studies using in vivo recording techniques would be needed to assess whether Slu participates in tectal motion detection and/or modulates tectal receptive fields.

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