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# Morphological and Biophysical Properties of the Cells Involving the Reflexive Orienting Responses of the Eye, Head and Body

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**Abstract:** The intrinsic membrane properties of the neurons in the cortices of the inferior colliculus were studied in 300- $\mu$ m transversal slices. The membrane properties, including the sub- and superthreshold, of the neurons were determined by intracellular stimulations with current injections. Of 49 intracellularly recorded neurons, 21 were successfully stained with intracellular biocytin injection. Morphologically all of the 21 neurons were identified as multipolar. Physiologically three firing patterns (onset, regular and adapting) were identified on the basis of response patterns to depolarizing current injections. Onset neurons had a non-linear, and regular and adapting neurons had linear current-voltage relationships. In conclusion, with the multipolar cell morphology and three physiological firing patterns, these neurons may be playing important roles in the processing of sound information mediating the reflexive orienting responses of the eye, head and body.

**Key Words:** Neuron, morphology, microelectrode, biocytin, in vitro.

## Göz, Baş ve Vücutun Pozisyonunu Sese Refleks Olarak Düzenleyen Sinir Hücrelerinin Morfolojik ve Biyofiziksel Özellikleri

**Özet:** Collicular inferiore'nin kabuk bölgesinde bulunan sinir hücrelerinin temel zar özellikleri, transversal olarak alınan 300  $\mu$ m kalınlığındaki kesitlerde çalışıldı. Sinir hücrelerin eşik değer altı ve üstü zar özellikleri, hücrelere akım enjeksiyonu yapılarak belirlendi. Zar özellikleri belirlenen 49 sinir hücresinden 21 tanesi, hücre içine biyosaytin enjekte edilerek boyandı. Boyanan hücrelerin hepsi morfolojik olarak multipolar şekilli idi. Fizyolojik olarak ise, zar özellikleri belirlenen bütün hücrelerde, depolarize edici akım enjeksiyonlarına karşı oluşan yanıt desenlerine göre onset, regular ve adapting olmak üzere üç ateşleme deseni (firing pattern) karakterize edildi. Onset ateşleme desenine sahip olan sinir hücreleri, linear olmayan; regular ve adapting sinir hücreleri ise linear olan akım-voltaj eğrilerine sahip olduğu saptandı. Sonuç olarak multipolar şekilli morfolojileri ve üç tip fizyolojik ateşleme deseni ile bu sinir hücreleri, göz, baş ve vücudun sese refleks olarak verdiği tepkiye aracılık eden ses bilgisinin işlenmesinde önemli rol oynadığı kanısına varıldı.

**Anahtar Sözcükler:** Sinir hücresi, morfoloji, mikroelektrod, biyocytin, in vitro.

## Introduction

The rat inferior colliculus (IC) consists of three regions: the central nucleus (CNIC), external cortex (ECIC) and dorsal cortex (DCIC) (1,2). The CNIC is the main integrative nucleus for the inputs from lower auditory nuclei in the IC. It is well characterized by a laminar organization formed by the flattened dendritic trees of the principle cells of the CNIC (3,4). The cortices are the shell of the IC encapsulating the CNIC. The ECIC is a laminar structure formed by three layers (1). The ECIC sends fibers to the medial and preolivary regions of the medial geniculate body (MGB), contralateral superior

colliculus (SC), pontine nuclei, periaqueductal gray, cerebellum, nucleus sagulum, reticular pontis oralis, the ventral nucleus of the trapezoid body and the cochlear nucleus. The DCIC sends fibers to the deep nucleus of the MGB (4,5). It has been suggested that the projections to the SC, the periaqueductal gray, and reticular pontis oralis allow the external cortex to serve as a link between sensory (auditory) and motor pathways (5). The visceral or emotional responses and the reflexive orientation responses of the pinea, eye, head and body are initiated in response to acoustic stimuli (6-8). The cortices function within the extralemniscal system.

Previous Golgi staining studies shed some light on the types of cells in the CNIC and the 2-D arrangements of these cell types (3). However, Malmierca et al. (2) demonstrated that 2-D observations of the neuronal arbors might not strictly give adequate information for precise recognition of cell types and their relative organization. Thus, in this study our aim was to characterize the morphology of neurons in the cortices of IC neurons with intracellular staining and then to analyze them with the aid of a 3-D analysis method. Simultaneously we aimed to couple the morphological analysis with recording of the intrinsic membrane properties.

### Materials and Methods

*Slice preparation of the IC:* For intracellular staining, slices from rat IC were used. Wistar rats of either sex between 13 to 16 days of age were used to minimize age-related and developmental variations (9). Immediately after sacrifice by cervical dislocation, the head was immersed in cold (4-8 °C) oxygenated Na-free artificial cerebrospinal fluid (S-aCSF) (sodium ions were replaced by sucrose on an equimolar basis) (10). The whole brain was rapidly removed and placed in freshly oxygenated cold S-aCSF. The brain stem containing the IC and SC was freed from the rest of the brain by cutting transversely. Another transverse cut was made in the remaining block between the IC and the cerebellum (caudal to the IC). The specimen was mounted with a cyanoacrylate glue, with the superior colliculus end down, onto a mounting block. Then 300 µm thick slices were cut using a series 1000 Vibratome filled with cold continuously oxygenated S-aCSF (3-8 °C). The first three slices containing the IC were transferred into a storage chamber containing fresh continuously oxygenated S-aCSF. The slices were kept in S-aCSF for at least 30 minutes to allow recovery from any surgical trauma. An appropriate slice was then transferred to a submerged-type recording chamber devised by Oertel (11). The slice was perfused with artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl, 124; KCl, 5; KH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.4; MgSO<sub>4</sub>, 1.3; NaHCO<sub>3</sub>, 26; glucose, 10; saturated with 95% O<sub>2</sub>/5% CO<sub>2</sub> gas; pH 7.4 at a rate of 6-7 ml/min. All chemicals were obtained from BDH and were of AnalaR grade. Recordings were performed at 35 °C.

*Intracellular biocytin labeling:* At the end of each successful recording, biocytin was injected into the impaled cell by iontophoresis using depolarizing current pulses of 1 nA amplitude with a duty cycle of 200 ms for durations varying between 3 and 30 minutes. The position of the injected neuron was noted on a sketch of the slice. Following the injection the slice was removed from the recording chamber and then fixed for at least 24 hours in 2% glutaraldehyde/2% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). For cryoprotection, the slices were then left in 30% sucrose for one day and were then cut into 50-60 µm sections on a freezing microtome. The sections were incubated for 2-3 hours at room temperature or overnight at 4 °C with 20 µl avidin D-HRP (Vector Laboratories) in 10 ml sodium phosphate buffer (0.1 M, pH 7.4) with 1% Triton X-100. Sections were reacted using the DAB-nickel/cobalt intensification method (12), then mounted onto gelatin-coated slides, and finally dehydrated, counterstained with neutral red and coverslipped. The position of the cell body in respect to the divisions of the IC was marked in the drawn sketch using a camera lucida attached to a Zeiss microscope. 3-D reconstructions of biocytin labelled cells were made using a NeuroLucida (MicroBrightField Inc. 74 Hegeman Ave., Colchester, VT 05446 USA), which allows an image to be viewed through the microscope eyepieces. Simultaneously a view of the computer screen is superimposed on the real image. The neurons reconstructed were displayed on an IBM compatible personal computer using Neurorotate, which was supplied by the same company.

*Intracellular recording:* Recording electrodes were freshly made from borosilicate glass capillary with a 1 mm external and 0.58 mm internal diameter (Clark Electromedical Instruments). Intracellular recordings were made with microelectrodes filled with 2% solution of biocytin in 2 M K-acetate, buffered to pH 7.4 with acetic acid. For stable recordings, the tip resistance of the most suitable microelectrodes ranged between 100 and 160 MΩ. Only microelectrodes with the ability to pass currents of 2 nA were used. Current-clamp experiments were carried out with an Axoclamp-2A amplifier (Axon Instruments, Inc., Burlingame) in bridge mode. Intracellular recordings were carried out if the cell had resting membrane potentials more negative than -50 mV. Generated data were filtered at 2000 Hz with a two-channel low pass variable filter (Kemo). Current and

voltage records were sampled at 16 kHz and were digitized online using a 16 bit A/D converter and stored on a computer for analyses. The intrinsic membrane properties of the IC neurons were studied using sets of depolarizing and hyperpolarizing current pulses with varying durations at normal resting membrane voltages. Acquisition and off-line analyses of digitized data were performed using software. The capacity compensation was used maximally without obtaining oscillation. The bridge balance was carefully adjusted. The scheme and terminology of Faye-Lund and Osen (1) were used here. When possible, the results are given as mean  $\pm$  standard errors (SE); N is the number of neurons.

## Results

Twenty-one rats were used, and 21 neurons in the cortices of the IC were successfully stained intracellularly with biocytin, and the intrinsic membrane properties of 49 neurons were recorded.

*Standard microscopy of somata and dendritic arbors:* The sizes of the cell bodies varied from 11 x 16 to 21 x 29  $\mu\text{m}$  and their shapes varied from spheroidal to triangular and ovoid polygonal. The variabilities in size and shape were not correlated with the position in the cortices. The origins of the dendrites were not at the two poles of the neurons but on all sides and the soma gave rise to four to eight dendrites branching repeatedly. A camera lucida drawing of a multipolar cell is shown in Fig. 1. The root of the dendrites were as thick as 5  $\mu\text{m}$ . They were smooth and irregular in thickness, i.e., tapering as they headed away from the cell body, contrasting with the regular thickness of the axon. Therefore, one can distinguish the axon and dendrites from each other. Tufting was rarely seen at dendritic terminations. Mostly intermediate and terminal segments of the dendrites were generally covered with varying densities of spine-like appendages of different lengths (ranging from 1 to 4  $\mu\text{m}$ ). The axon originated from the cell soma, and, rarely, from the largest basal dendrites. The axons of several neurons gave off collaterals, forming a dense plexus.

*Computer-aided analyses of dendritic arbors:* Three-dimensional analysis of the dendritic arborization of the cells in the cortices revealed that only one type of neuronal cell existed, which is multipolar and non-oriented (Fig. 2). The dendritic arborization mostly

extended in all directions, generally displaying a clear multipolar and unoriented shape when viewed in 2-D and also in 3-D observations. The dendritic fields of the neurons extended to up to 780  $\mu\text{m}$  in length and the ratio of length over width varied from 1.6 to 3.7. The dimensions, length, width and thickness were measured on the screen when rotated suitably. The thickness and width were more than 140 and 210  $\mu\text{m}$  and the averages were  $212.3 \pm 11.2$  and  $332.4 \pm 17.3$   $\mu\text{m}$  ( $N = 21$ ), respectively.

*Electrophysiology:* About 25% of the neurons showed spontaneous activity consisting of action potentials (APs), EPSPs and less frequently IPSPs. The recordings lasted for about 20 minutes to 3 hours. These cells had resting potential around  $-60$  mV (ranging between  $-50$  and  $-73$  mV; mean  $-59$  mV) and the input resistance, calculated from a linear fit of the negative section of the I-V relationship of the peak voltage responses near rest, was  $71.4 \pm 6$   $\text{M}\Omega$  ( $n = 28$ ). The mean membrane capacitance and time constant of the neurons were  $86.4 \pm 9.3$  pF ( $n = 25$ ) and  $5.4 \pm 0.8$  ms ( $n = 20$ ), respectively. The AP amplitudes varied between 35 mV and 80 mV.

The membrane properties of the neurons in the cortices of the IC were studied using depolarizing and hyperpolarizing current pulses. In response to current pulses, the neurons in the cortices were diverse in superthreshold responses. Two types of neurons with regular (17/49) (Fig. 3C) and adapting firing patterns (28/49) (Fig. 3B) were common. An onset firing pattern was rarely observed (4/49) (Fig. 3A). Regular neurons fired trains of APs with a constant interspike interval to current injection. On the other hand, the adapting neurons fired APs with increasing interspike intervals throughout the current injection. Onset neurons fired only one AP at the onset of a stimulus irrespective of the amplitude of the current injected. In neurons with regular and adapting firing patterns, in response to increased levels of depolarizing current, the number of APs increased and the interspike interval decreased but the AP duration remained constant. Seven regular and 11 adapting neurons showed rebound spiking following a period of hyperpolarization of sufficient amplitude. In response to hyperpolarizing current pulses, only three regular neurons and the majority of adapting neurons showed anomalous rectification but in no onset neurons was anomalous rectification seen. In onset neurons, the I-

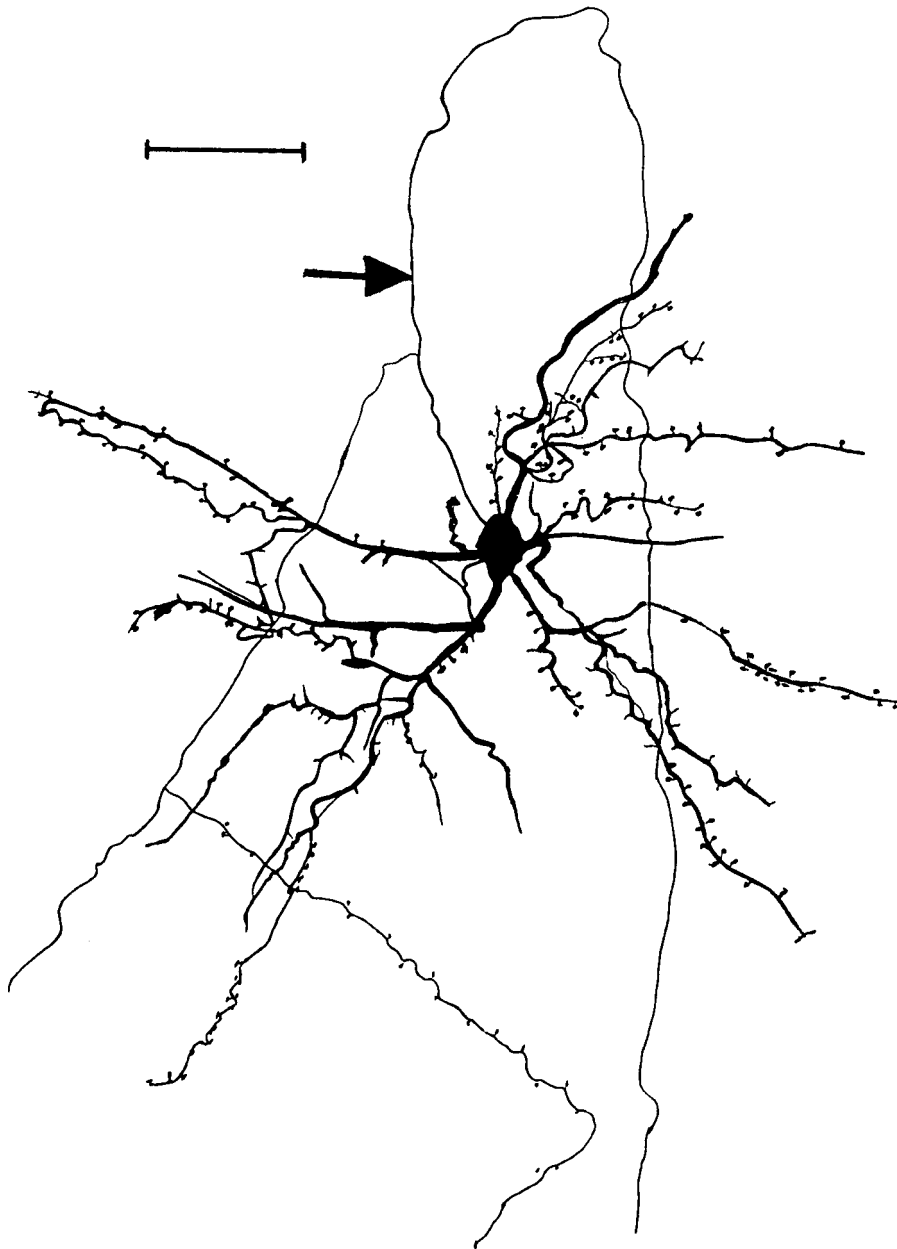


Figure 1. Camera lucida reconstruction of a biocytin-stained multipolar neuron. The axon of the cell is shown by the arrow and note that the axon has a uniform thickness, whereas the dendrites were smooth and irregular in thickness, i.e., tapering as they headed away from the cell body. Calibration: 100  $\mu$ m.

V relationship was clearly nonlinear over a depolarizing current range, whereas both regular and adapting neurons appeared to have linear I-V curves.

### Discussion

*Morphology:* The morphology of the neurons revealed by intracellular biocytin staining in the cortices of the rat

IC largely resembles those described on the basis of Golgi material in the rat IC (1-3). The cells generally appeared to fit the description of stellate neurons described by Morest and Oliver (3) in 2-D observations. Malmierca et al. (2) described stellate cells as being oriented multipolar like cells, i.e., no clear orientation. In this material, however, the dendritic arbor tended to occupy a larger area. The differences could partly be due to the different

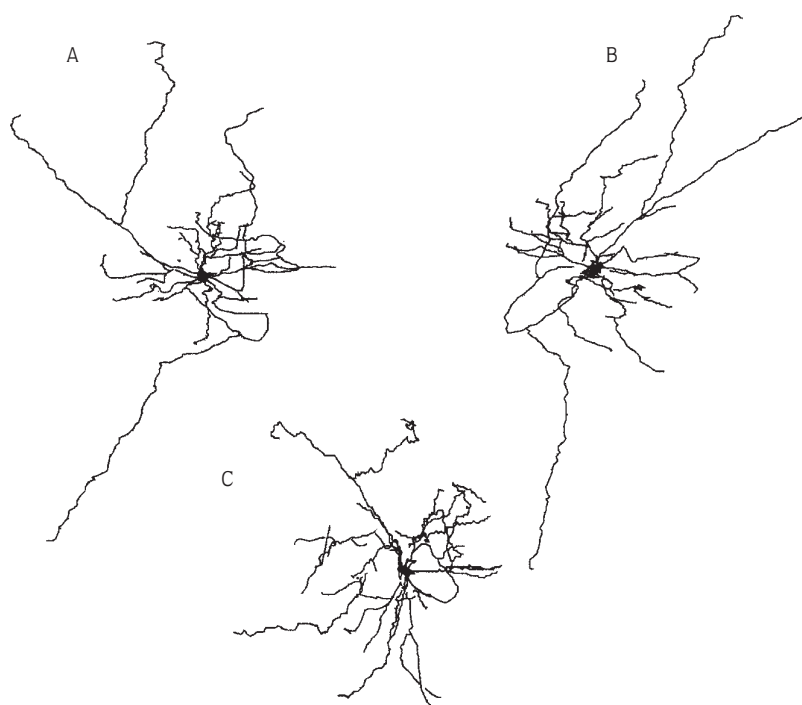


Figure 2. 3-D reconstruction of a cell in the cortex. The dendritic arborization of the neuron looks non-orientated in all three views. A: The neuron is shown as drawn in the transversal plane. B: the neuron was rotated until the best view of the width was obtained. C: the neuron was rotated until the least thickness was obtained. Calibration: 100  $\mu\text{m}$ .

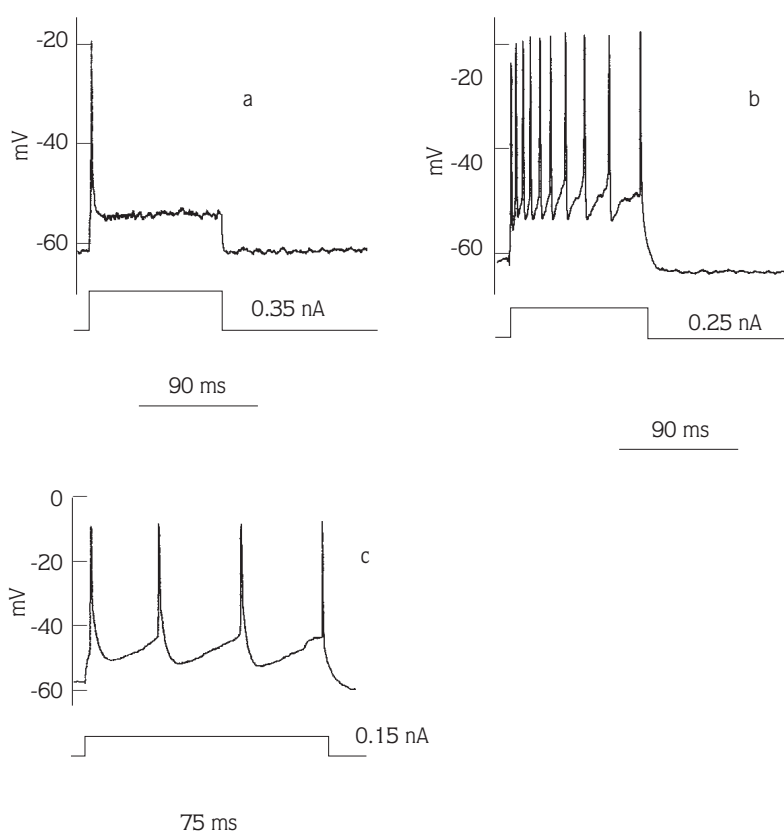


Figure 3. Characteristic firing behaviors of onset (A), adapting (B), regular (C) A: This neuron fired only one AP in response to depolarizing current steps. B: The APs exhibited considerable spike-rate adaptation. C: Response of a regularly firing neuron, illustrating a regular response pattern with approximately equal interspike intervals.

methods used; large cells are more likely to be stained by the intracellular staining technique because there is more chance of hitting large cells and also stable recording is probably from large cells.

Another intracellular study by Wagner (13) in the mouse, in which the anatomy of CNIC neurons has been studied, showed that the cells he stained with biocytin injection seem to be similar to the multipolar cells. However, one would expect that the orientation of the CNIC neurons' dendritic arborizations should display an elongated and flattened shape, since it is true for CNIC of the rat and cat (2,14,15). Species differences is the most likely explanation for different results. It is also possible that the cells in his report could be situated in the pars lateralis subdivision of the central nucleus of the IC as defined by Oliver and Morest (15). This is an interesting possibility, since their cells look much like the cells stained in this study; thus they might be from the same region. Since Malmierca et al. (2) showed that this subdivision is populated by a different cell type from those seen in the central nucleus they suggested that this region is distinct from the central nucleus and should be included in the external cortex. Therefore they might, in fact, be multipolar neurons in the ECIC. The morphology of the cell that they described is similar to that of the cells described in this study. All the neurons sampled from cortices as defined by Faye-Lund and Osen (1) differed from those sampled flat cells in the CNIC in many respects (2,14). The intracellular study by Smith (16) confirms the morphological findings of our study.

*Physiology:* The neurons in the cortices of the IC may be divided into three groups (onset, regular and adapting) on the basis of response patterns to depolarizing current

injections. Onset neurons with non-linear and regular and adapting neurons with linear I-V relationships were described in the mouse (13) and rat central nucleus of the IC (17-19) and in the cortices of rat IC (16). The results presented here are consistent with these early reports. None of the three firing patterns corresponds to a separate morphology, since only multipolar cells with uniform morphology were observed in this study. However, it is possible that the cell in the cortices of IC could be classified in more than one group if more criteria apart from dendritic arborization had been used, for which a large number of samplings has to be carried out. In other auditory brain stem nuclei, the onset neurons appear to have a non-linear I-V relationship and this non-linearity has been associated with low-threshold outward potassium currents (20,21). Similarly, in the CNIC, the onset firing has also been reported to be associated with the low-threshold outward current (19). It is also possible that the non-linearity may be due to the low-threshold outward potassium currents in the cortices of the IC, which is activated at potentials close to the resting potential (22,23).

In conclusion, the visceral or emotional responses and the reflexive orientation responses of the pinna, eye, head and body initiated in response to acoustic stimuli are processed by the neurons with multipolar morphology and with different firing patterns including onset, regular and adapting.

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