Neuroscience 136 (2005) 843–863

CONVERGENCE OF AUDITORY-NERVE FIBER PROJECTIONS ONTO GLOBULAR BUSHY CELLS

G. A. SPIROU,[a]* J. RAGER[a] AND P. B. MANIS[b]

[a]*Sensory Neuroscience Research Center, West Virginia University School of Medicine, PO Box 9303 Health Sciences Center, One Medical Center Drive, Morgantown, WV 26506-9303, USA*

[b]*Department of Otolaryngology/Head and Neck Surgery, University of North Carolina at Chapel Hill, 1123 Bioinformatics Research Building CB#7070, Chapel Hill, NC 27599-7070, USA*

Abstract—Globular bushy cells are a key element of brainstem circuits that mediate the early stages of sound localization. Many of their physiological properties have been attributed to convergence of inputs from the auditory nerve, many of which are large with complex geometry, but the number of these terminals contacting individual cells has not been measured directly. Herein we report, using cats as the experimental model, that this number ranged greatly (9–69) across a population of 12 cells, but over one-half of the cells (seven of 12) received between 15 and 23 inputs. In addition, we provide the first measurements of cell body surface area, which also varies considerably within this population and is uncorrelated with convergence. For one cell, we were able to document axonal structure over a distance greater than 100 μm, between the soma and the location where the axon expanded to its characteristic large diameter. These data were combined with accumulated physiological information on vesicle release, receptor kinetics and voltage-gated ionic conductances, and incorporated into computational models for four cells that are representative of the structural variation within our sample population. This predictive model reveals that basic physiological features, such as precise first spike latencies and peristimulus time histogram shapes, including primary-like with notch and onset-L, can be generated in these cells without including inhibitory inputs. However, phase-locking is not significantly enhanced over auditory-nerve fibers. These combined anatomical and computational approaches reveal additional parameters, such as active zone density, nerve terminal size, numbers and sources of inhibitory inputs and their activity patterns, that must be determined and incorporated into next-generation models to understand the physiology of globular bushy cells. **© 2005 IBRO. Published by Elsevier Ltd. All rights reserved.**

*Corresponding author. Tel: +304-293-2457; fax: +304-293-7182.
E-mail address: gspirou@hsc.wvu.edu (G. Spirou).

Abbreviations: ANF, auditory nerve fiber; AVCN, anteroventral cochlear nucleus; AZ, active zone; CV′, coefficient of variation (corrected); EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GBC, globular bushy cell; g_{KHT}, high-threshold potassium channel conductance; g_{KLT}, low-threshold potassium channel conductance; g_{NA}, sodium channel conductance; m, number of active zones; ISIH, interspike interval histogram; LS, large spherical vesicle; MAC, mitochondrion-associated adherens complex; mEPSC, miniature excitatory postsynaptic current; MNTB, medial nucleus of the trapezoid body; *N*, number of auditory nerve fibers; PL, pleomorphic vesicles; P_r, vesicle release probability per active zone; Pri-N, primary-like with notch; PSTH, peri-stimulus time histogram; SBC, spherical bushy cell; SS, small spherical vesicle.

Key words: auditory, sound localization, endbulb, synaptic integration, electron microscopy, computational modeling.

Bushy cells constitute one of the principal cell types of the cochlear nucleus (Ramón y Cajal, 1911; Osen, 1969; Brawer et al., 1974) and exhibit specialized physiological signatures (Rhode and Smith, 1986; Smith and Rhode, 1987; Friauf and Ostwald, 1988; Young et al., 1988; Blackburn and Sachs, 1989; Spirou et al., 1990; Smith et al., 1991) that preserve many of the response characteristics of auditory nerve fibers (ANF). These characteristics of ANFs include an increased probability to generate action potentials at the onset of a sound, random intervals between spikes during a stimulus sustained at constant intensity, and phase-locking with high temporal precision to low frequency dynamics of acoustic stimuli (Kiang et al., 1965; Johnson, 1980). Bushy cells provide massive convergent projections to binaural neurons in the core of the brainstem, and thereby mediate the initial stages of sound localization (Stotler, 1953; Warr, 1972; Smith et al., 1993; Beckius et al., 1999). The similarity of bushy cell and ANF physiology derives in part from a combination of synaptic organization, whereby ANFs deliver large terminals onto bushy cell somata (Harrison and Irving, 1965; Brawer and Morest, 1975; Gulley et al., 1978; Cant and Morest, 1979; Lorente de Nó, 1981; Tolbert and Morest, 1982b), and intrinsic ionic conductances of bushy cells, which favor rapid resetting of the membrane potential between synaptic inputs (Oertel, 1983; Manis and Marx, 1991).

Two types of bushy cells can be distinguished anatomically based on cytological features and differences in synaptic input from ANFs, and are termed spherical or globular, according to the geometry of their cell bodies (Osen, 1969; Brawer et al., 1974). Spherical bushy cells (SBCs) are contacted by two to four large, complex nerve terminals called endbulbs of Held, each of which contains multiple synaptic sites (Cant and Morest, 1979; Sento and Ryugo, 1989; Ryugo and Sento, 1991). Globular bushy cells (GBCs) are contacted by a larger number of inputs, many of which have complex geometry but that are, on average, smaller than endbulbs and have been termed modified end bulbs (Harrison and Irving, 1965; Fekete et al., 1984; Rouiller et al., 1986; Redd et al., 2000).

Despite their general similarities, sound-evoked responses of GBCs can be distinguished from those of ANFs due, in part, to convergence of multiple ANF inputs onto single cells. GBCs generate a precisely timed action potential at the onset of a sound, followed by a decrease in firing probability that often appears as a notch (Bourk, 1976) in a peri-stimulus time histogram (PSTH). Another

0306-4522/05$30.00+0.00 © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2005.08.068

distinction, also shared by some SBCs, is improved phase-locking relative to ANFs in response to low frequency sound (Joris et al., 1994a,b). Existing models designed to account for these and other response properties of GBCs employ varying combinations of suprathreshold and subthreshold inputs, the latter of which must arrive coincidently to drive activity (Rothman et al., 1993; Joris et al., 1994a,b; Rothman and Young, 1996; Kuhlmann et al., 2002).

Despite the importance of the number of convergent ANF inputs to understanding the physiology of GBCs, this quantity has not been measured directly. Estimates for the number of convergent inputs onto GBCs have been based on intracellular labeling studies where numbers of different ANF terminal types were quantified within spontaneous rate categories (Fekete et al., 1984; Rouiller et al., 1986; Liberman, 1991, 1993), and scaled using estimates for total numbers of ANFs and cochlear nucleus cell types (Spirou et al., 1990; Liberman, 1991). The studies by Ryugo and colleagues (Fekete et al., 1984; Rouiller et al., 1986) provided criteria to identify and quantify numbers of modified end bulbs associated with individual ANFs. From these data we estimated that 17 modified end bulbs terminate onto each GBC cell body (Spirou et al., 1990). Liberman (1991) observed a continuum of ending complexity for ANF terminals, ranging from boutons to large, complex terminals, in regions of the cochlear nucleus that contain GBCs. He estimated, using light microscopy, that about 50 ANF terminals spanning this range of size and complexity contacted GBC somata.

Determination of the number of terminals contacting GBC somata, using light microscopy, can be confounded if terminals contact dendrites of nearby cells rather than the cell body of a neuron located near the terminal. We undertook a serial section method to count ANF inputs in electron micrographs onto individual GBCs and verify the excitatory nature of these terminals using ultrastructural criteria. We employed an approach, alternating ultrathin and semithin sections, to permit the counting of inputs from myelinated axons onto multiple GBCs. Examination of ultrathin sections also made possible determination of the percent somatic surface area contacted by excitatory and inhibitory terminals. Using these data, we have provided the first estimates for the number of inhibitory terminals contacting individual GBC cell bodies. A key result of these investigations is that the population of GBCs, generally considered to be a homogeneous cell group, exhibits large variation in the numbers of excitatory and inhibitory inputs and cell size, and that these parameters vary independently.

To understand how the convergence of ANFs can affect spike patterns of GBCs, we implemented a detailed model for four GBCs, selected from and representative of the population studied structurally, and analyzed their responses to auditory nerve-like inputs. The model combines structural data from this study with information from recent investigations of physiological features such as ion channel kinetics, and synaptic function including vesicle release probability, synaptic depression, and receptor kinetics. The modeling suggests constraints on structural features, such as active zone (AZ) density, that have not been quantified experimentally and, when driven using realistic ANF input patterns, predicts spike trains of GBCs, in response to tones at characteristic frequency, that are similar to those recorded *in vivo*.

EXPERIMENTAL PROCEDURES

Electron microscopy

Two adult cats, age 12 and 15 months, bred and raised in a clean environment to be free of ear infections, were anesthetized with Nembutal (35 mg/kg). They were perfused transcardially with a calcium-free Ringer's solution followed by a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.12 M phosphate buffer, pH=7.3. All protocols were approved by the Institutional Animal Care and Use Committee of the West Virginia University School of Medicine, conform to international guidelines on the ethical use of animals and minimize suffering. Our combination of experimental and modeling procedures serve to minimize the number of animals used in these studies. Each brainstem was cut into 200 μm thick sections in the coronal plane, postfixed with 1% osmium tetroxide, stained with 2% uranyl acetate, dehydrated, and flat-embedded in Epon. Tissue containing the cochlear nucleus was blocked and re-embedded. The tissue was trimmed to include the lateral region of the nerve root (Tolbert and Morest, 1982a) that has been shown to contain a nearly homogeneous population of GBCs (Fig. 1A, B). This region of the nucleus contained cells having low characteristic frequency (Bourk et al., 1981).

Our first approach was to prepare series of alternating 1 μm semithin and two to four ultrathin sections (70 nm thickness) to assess our ability to detect myelinated ANF inputs to GBCs through a large thickness of tissue. We found that ANF inputs that were evident in ultrathin sections could not be detected reliably in semithin sections. Therefore, we developed an approach to detect ANF inputs using ultrathin sections. From existing series of serial sections in our laboratory (Rowland et al., 2000), we found that we could detect cellular processes whose caliber was approximately equal to the spacing between ultrathin sections selected from this series, since rarely did the processes lie exactly parallel to the section plane. As described in the Results section, we determined that the thinnest myelinated axons in the region of cochlear nucleus under study were about 0.7 μm thick. In a second animal, series of alternating sequences of two ultrathin (70 nm thickness each) and one semi-thin section (0.5 μm) were prepared (Fig. 1C), encompassing a total tissue thickness of 31 μm. A sequence of semi-thin sections was collected preceding and following this series to assess the completeness of sectioning through each cell. A section near the middle of the series was viewed, and cells that were judged by the presence of a large nucleus to be centered in the series of ultrathin sections were chosen for analysis. GBCs typically have one primary dendrite that extends 10–15 μm from the cell body prior to branching. Our intention was to achieve a complete or nearly complete representation of the cell body and the initial 10 μm of the proximal dendritic tree. Twelve cells were tracked through this series of tissue sections. One entire cell body with proximal dendrite and greater than 90% of another four cells were contained within the series. The completeness of reconstruction was less than 80% (78%) for only one cell. Structural features that were quantified in the second animal were verified qualitatively to be present in the series of sections from the first animal.

Ultrathin sections were viewed and photographed using a JEOL 1010 microscope (Tokyo, Japan) operated at 80 kV. Each GBC was photographed at 1500–2000× to capture the entire cell body and proximal dendrite at as high a resolution possible in a single micrograph. From these images we identified heminodes,

Download English Version:

https://daneshyari.com/en/article/9425576

Download Persian Version:

https://daneshyari.com/article/9425576

Daneshyari.com