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Response patterns to sound associated with labeled globular/ bushy cells in cat

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Abstract

The mammalian cochlear nucleus (CN) consists of a diverse set of neurons both physiologically and morphologically that are involved in processing different aspects of the sound signal. One class of CN neurons that is located near the entrance of the auditory nerve (AN) to the CN has an oval soma with an eccentric nucleus and a short-bushy dendritic tree and is called a globular/bushy cell (GBC). They contact the principal cells of the medial nucleus of the trapezoid body (MNTB) with the very large calyx of Held that is one of the most secure synapses in the brain. Because MNTB cells provide an inhibitory input to the lateral superior olive, a structure purported to play a role in lateralizing high frequency sounds, GBC physiology is of great interest. Results were obtained with intracellular recording and subsequent labeling with neurobiotin of 32 GBCs along with a number of cells characterized extracellularly as likely GBCs in the cochlear nucleus (CN) of cat. Their poststimulus discharge response pattern to repeated tones varies from a primarylike pattern, i.e., similar to the AN, to a primarylike pattern with a 0.5 to 2 ms notch after the initial spike, to an onset pattern with a low-sustained rate. They can represent low frequency tones and amplitude modulated signals exceptionally well with a temporal code.

Introduction

The mammalian cochlear nucleus (CN) contains a set of physiologically and morphologically diverse neurons that are involved in processing different aspects of the sound signal. It provides the substrate for selectively enhancing information that upstream auditory centers require to perform their functions, whether they are involved with sound localization, sound identification, or signaling “escape now or forever be silent”. The number of proposed cell types in the CN ranges from nine (Osen, 1969) to 22 (Brawer *et al*, 1974) to 55 (Lorente de Nó, 1981) with the difference largely resulting from the difference in Nissl versus Golgi staining techniques, and presumably because observers can be considered either “lumpers” or “splitters” when classifying morphologies. A large range of physiological cell types has also been proposed based on the post-stimulus time histogram (PSTH) formed in response to relatively short tones (25 – 50 ms) at the characteristic frequency (CF) of the neuron. For example, Pfeiffer (1965) identified onset, primary-like and chopper responses in the ventral cochlear nucleus. Subsequently, PSTH response patterns have been greatly elaborated (Bourk, 1978; Blackburn and Sachs, 1989), and the relation of these PSTH patterns to the underlying morphology has been a subject of study. The introduction of intracellular labeling of physiologically characterized cells provided a firm basis for correlating physiological and morphological cell types (e.g., Rhode *et al*, 1983; Rouiller and Ryugo, 1984). Intracellular

studies in brain-slices resulted in the biophysical characterization of several types of ion channels for many CN cell types, which also aided in the search for physiological-morphological correlations (e.g., Oertel, 1983; Manis and Marx, 1991; Rothman and Manis, 2003a; Cao *et al*, 2007).

There are several PSTH-defined cell categories that display a marked onset response characterized by a high ratio of initial to steady-state discharge rate. These include 1) the onset-ideal (OnI, Godfrey *et al*, 1975) PSTH response type that originates from octopus cells in the postero-ventral cochlear nucleus (PVCN) as were morphologically characterized by Osen (1969); 2) onset-choppers (OnC) identified as large multipolar cells in the PVCN by Smith and Rhode (1989); and 3) primarylike-with-notch (PLn) units, Onset with an L-Shaped PSTH (OnL) units, and primarylike (PL) units. PLn, OnL and PL units arise from globular/bushy cells (GBCs), which exhibit a characteristic eccentric nucleus and are mostly located in the nerve root area (NRA) of the CN which Osen (1969) named the central region of the ventral cochlear nucleus. The OnL pattern in cat has been described as having only an onset spike at low intensities that transitions to the OnL pattern at higher intensities (Godfrey *et al*, 1975). In the guinea pig anteroventral cochlear nucleus (AVCN), OnL patterns have also been associated with stellate cells (Winter and Palmer, 1995; Arnott *et al*, 2004).

An intriguing feature of these onset-PSTH types is that each cell type displaying such responses either appears to be inhibitory or to excite an inhibitory postsynaptic neuron (see review: Cant and Benson, 2003). OnC's are hypothesized to provide glycinergic wideband-inhibitory inputs to the DCN and PVCN, and project to the contralateral CN (Smith *et al*, 2005, 1989; Arnott *et al*, 2004). OnI (octopus cells) units project to the contralateral ventral nucleus of the lateral lemniscus (VNLL) where they terminate in a calyx ending on cells that are glycinergic (e.g., Adams and Wenthold, 1987; Saint Marie *et al*, 1997;) and project to the ipsilateral inferior colliculus (IC). PLn/OnL cells have a major projection to the contralateral MNTB where they synapse on glycinergic inhibitory principal cells that project to the LSO (Bledsoe *et al*, 1990, Spirou *et al*, 1990). This connection plays a prominent role in creating an interaural level difference (ILD) coding observed in the response of LSO neurons that are used predominantly to localize high frequency sounds. MNTB neurons also project to the VNLL, where they terminate in calyceal-like endings (Adams and Wenthold, 1987). VNLL is part of a monaural ascending system purported to play a role in temporal processing of signals (Covey and Casseday, 1991).

These onset unit types typically display relatively short first-spike latencies with nearly a 100% rate-of-occurrence of the initial spike in response to short CF tones sufficiently above threshold. Each onset unit type has voltage-sensitive conductances (Trussell, 1997) and superb temporal response properties, i.e. precise firing to a particular phase of a sinusoidal stimulus, termed phase locking. This phase locking can exceed that of auditory nerve fibers (ANFs) at frequencies less than 1 kHz (Joris *et al*, 1994a). A consequence of such fast voltage-sensitive conductances is that converging inputs must occur within a relatively small temporal window in order to fire the postsynaptic element, when individual excitatory postsynaptic potentials (EPSPs) are not capable of discharging the postsynaptic element (McGinley and Oertel, 2006). In other words, these onset neurons behave as coincidence detectors. In engineering terms, they differentiate the signal, thereby emphasizing changes in the signal rather than coding the steady-portion (Ferragamo and Oertel, 2002). Another characteristic of units having an onset PSTH is that they generally have a large number of inputs, possibly ranging as high as 100 (Golding *et al*, 1995; Spirou *et al*, 2005).

OnI and OnC's units in the PVCN have large dendritic trees that intersect with a substantial portion of the tonotopic afferent field of ANFs having a range of CFs. This results in broader filters, i.e. lower Q_{10} 's ($Q_{10} = \text{CF}/\text{bandwidth at 10 dB above threshold}$), than other cell types

in the CN. In contrast, PLn units may obtain their entire primary afferent input in the form of 'modified endbulbs of Held' from ANFs with nearly similar CFs, and their frequency selectivity (Q_{10}) is the same as found for ANFs (Rhode and Smith, 1996a). Modified endbulbs are a smaller variant of the large 1 or 2 endbulbs that contact large spherical/bushy cells in the AVCN (Rouiller *et al*, 1986; Liberman, 1991). In the latter instance, they are assumed to provide secure transmission of the afferent activity to the post-synaptic element. Modified endbulbs converge on GBCs with several to more than 60 endings (Liberman, 1993; Spirou *et al*, 1990; Spirou *et al*, 2005).

A possible outcome of a high degree of ANF convergence is that the GBC-PSTH response pattern could vary from cell to cell. Early attempts at structure/function correlation using intracellular injection of HRP (Rhode, *et al*, 1983; Rouiller and Ryugo, 1984) indicated that PL, OnL and PLn response patterns can arise from GBCs based on a relatively small number of observations in the NRA. One likely reason for this range of GBC-PSTH properties is that there are three 'distinct' populations of auditory afferents, i.e. ANFs with different spontaneous and driven spike activities (Liberman, 1978). Modeling studies demonstrated that in order to capture the range of PSTH responses seen, there is a requirement for numerous sub- and supra-threshold inputs, along with inhibitory inputs and a range of afferent spontaneous activities (Rothman *et al*, 1993).

Because each morphological category has a set of physiological response distributions that often overlap with those of another morphological category, it is difficult to definitively classify a unit based on the physiology when response patterns are not linked to an anatomically identified neuron. In the CN, several neuron types have onset PSTH patterns, a high degree of synchronization to low frequency tones, a range of spontaneous activity and similar maximum discharge rates. This study was undertaken to further clarify the correlation of structure and physiology of one of these cell types, the GBCs. 32 full or partially labeled GBCs with 15 PLn, 12 PL and 5 OnL PSTH response patterns were physiologically characterized and anatomically reconstructed after being labeled with neurobiotin.

Methods

Young adult cats of either sex ($n = 129$) were anesthetized with pentobarbital using a dose rate of 75 mg/kg. Additional doses (50 mg/kg) were administered to maintain the animal in a deeply areflexive state in accordance with NIH guidelines. The animal was placed on a respirator and expired CO_2 was maintained at 4%. All procedures were approved by the Animal Care and Use Committee of the University of Wisconsin.

An opening in the skull was made over the posterior fossa. The cerebellum was partially aspirated and the remainder was lifted and pushed anteriorly with small pieces of cotton to expose the CN. Two scales were positioned alongside the CN and were used to record X and Y co-ordinates of electrode placements. A 2% agar solution covered the CN to reduce pulsations. A plastic chamber was cemented over the opening in the skull and then filled with warm mineral oil. A glass cover was positioned on the chamber with high vacuum grease to create a hydraulically sealed chamber and reduce brain pulsation.

Intracellular labeling of physiologically characterized neurons was attempted using micropipettes filled with 1 M KCl and a 2% neurobiotin solution. At the end of a recording session, the animal was euthanized with pentobarbital, perfused with 1 liter of 1% paraformaldehyde and 1 liter of 2% glutaraldehyde. The brain was removed, and a cross-section of the brainstem containing the CN was cut and placed in 2% glutaraldehyde overnight. The following day the material was transferred to a sucrose solution to prepare it for frozen sectioning at 60 μm .

Zero to 8 neurons were labeled in any one experiment with an average of one per experiment. Penetrations were typically separated by 1mm to facilitate physiology-to-neuron correspondence. In total, over 140 cells of various morphologies were reconstructed in varying degrees of completeness, and 32 were GBCs. The 3-dimensional coordinates and size of the soma, axon and dendrites were captured and analyzed using the 3-D archiving program, Neurolucida (Microbrightfield). All drawings were made from coronal sections of the CN, and Neurolucida allowed viewing the morphology from any angle by computerized rotation of the neuron about each axis.

Prior to data collection, the acoustic system was calibrated with a 1.27cm Bruel & Kjaer condenser microphone/probe tube combination. An attempt was made to characterize each unit with the following paradigms: (a) the receptive field was determined using 50 ms tone pips with the intensity varied from 0 to 90 dB SPL in 10 dB increments. Frequency was varied in CF/10 to CF/20 Hz steps; (b) the PSTH at the CF was generated using 250 repetitions of a 50 ms tone presented every 105 ms, typically at 60 dB SPL; (c) an FM sweep response at a single intensity (usually 70 dB SPL) was collected; and (d) an amplitude modulated (AM) signal was presented to determine the temporal modulation transfer function (Rhode and Greenberg, 1994). The AM carrier was set to CF and the modulation depth was 100%. AM stimuli were 100 ms in duration, repeated 5 times every 200 ms, at stimulus intensities of 30, 50 and 70 dB SPL; modulation frequency was varied in 50 or 100 Hz steps over the range of interest. The complete stimulus series was rarely completed.

The first receptive field collected was limited to a single repetition of each frequency and intensity combination when the recording was intracellular. A one repetition paradigm was used because of the short intracellular holding times that were common in the cat. When the recording was stable, the receptive field paradigm was repeated with either 5 or 10 repetitions. One repetition made it difficult to always have enough spikes for reliable vector strength (VS) computations of phase locking so a Rayleigh coefficient of 0.001 was invoked as the criterion to determine if the phase locking was significant (Mardia, 1972). The Rayleigh coefficient for larger N is essentially a chi-square test of confidence of the VS. VS is calculated by considering each spike as a vector of unit length beginning at the origin (0,0) with a phase corresponding to where they occur relative to the stimulus phase. A resultant vector is calculated and normalized by the number of spikes (N), which yields the average phase and a VS. VS varies from 0.0 to 1.0, where 1.0 indicates perfect phase locking, i.e., all discharges occur in a single bin of a period histogram. VS was computed by selecting an analysis window (AW) equal to an exact multiple of the stimulus period ($T = 1/f$, f = frequency) within the last 90% of the response, i.e., for a 50 ms stimulus $AW = 5$ to NT ms, where N is the largest integer such that $NT < 45$ ms. This eliminates onset latency variability along with the problem of a non-integer number of periods of the stimulus, which would result in reducing the VS.

PLn patterns were identified by the presence of a 0.5 – 2 ms silent interval following the initial spike. An exception is that for low frequencies (i.e., < 1000 Hz), cells discharge in synchrony with the stimulus and the notch is not apparent. Because of the latter effect, cells with low CFs were labeled phase lockers by Joris *et al* (2004a). Here they will simply be considered low frequency PLn's.

The OnL pattern is somewhat problematic as this pattern varies somewhat from a prominent initial peak followed by a very even discharge rate throughout the rest of the response to one in which there is a slight dip in the firing rate immediately after the initial spike. There is almost never any rate adaptation in OnL responses after the initial spike, as is typically observed in PL responses. Blackburn and Sachs (1989) described two OnL patterns: (1) PSTH had no notch, a well-timed onset spike and a sustained firing rate < 100 sp/s and (2) a PSTH that had a gradual notch (> 1.5 ms) to distinguish it from the PLn pattern. However, there is no single set of

features that can be applied to distinguishing the two, as there is considerable overlap in steady-state firing rates, peak rates and spontaneous rates. OnL units have a bias toward higher peak-to-steady state firing rates than PLn units, though there is overlap even in this measure. As a result of this characteristic, the OnL classification is to be taken as a qualitative descriptor of the response pattern.

Of the 32 labeled GBCs, 14 were classified as PLn, 13 as PL and 5 as OnL. Recordings were also made from units that were likely GBCs characterized by their PSTH patterns and the fact that the electrode was in the NRA. Combining these two groups of units resulted in 44 PLn units, 54 PL units and 23 OnL units in this study.

Results

Poststimulus time histograms of Globular/bushy neurons

The range of response patterns obtained at CF and at 60 dB SPL from GBCs in the NRA include PLn, OnL and PL PSTHs (Figs. 1 & 2). GBCs phase lock to low-frequency stimuli, i.e., they discharge at a preferred phase of the stimulus, resulting in modes in the PSTH at multiples of the stimulus period T . Because the 0.5 to 2 ms notch is a result of the refractory period of the neuron (e.g., Rothman *et al*, 1993), when $f < 500$ Hz, T is longer than the notch precluding the use of this feature to classify the PSTH as PLn (Fig. 1A). PhL (phase-lockers) has been used to designate these low frequency patterns (Joris *et al*, 1994a). In this report, PSTH patterns are considered PLn when they are directly associated with a labeled GBC, or else if the VS is $> .9$ and the recording site was shown to be in the NRA. Phase-locked responses of GBCs remain prominent for frequencies < 2 kHz, as indicated by the modes in the PSTH at multiples of the stimulus period (panels A–D). The notch in the PSTH is apparent at above threshold intensities in panels B, C, D, F, and H and less so or even absent when CFs are greater than 10 kHz (e.g., panels G & I). Units with PLn responses have sustained discharge rates with a wide range of values (50 – 800 sp/s). For example, the sustained rate is so low in panels E and G that the pattern could as easily be classified as OnL; in contrast, the rate is very high (~ 550 sp/s) in panel H, so that unit is not a candidate for OnL classification. Some PSTHs for PLn units exhibit adaptation of the firing rate over a 5-10 ms interval after the initial spike as seen in panels B and D.

GBC PSTHs are usually PL at low intensities, as shown in Fig. 2A, and become PLn with increasing intensities. Additionally, latencies typically shorten and the notches lengthen with increasing intensity over the 90 dB range tested (Fig. 2 B-D).

Receptive fields

An auditory neuron receptive field is represented by a set of iso-intensity curves as a function of frequency. Representative receptive fields for five labeled GBCs, along with one unit that is likely a GBC that was recorded from intracellularly (panel D), are shown in Fig. 3. A common GBC receptive field feature is the limited extent of excitatory responses above CF (Figs. 3C–F). This could result if all afferent inputs had similar CFs or if there are inhibitory inputs in the high frequency region that effectively limit the spread of excitation. For example, in Figs. 3C and 3F there is a reduction of discharge rate below the unit's spontaneous rate (SR) with increasing intensity, as indicated by the arrows.

Some GBCs phase lock to nearly every stimulus cycle up to ~ 800 Hz (entrainment to the stimulus). If the unit has a high CF, this can result in an asymmetric shaped receptive field, i.e., higher spike rates at low frequencies than in the region of CF (> 2 kHz, Figs. 3C, 3D, 3E). In general, PLn units have a maximum steady-state discharge rate at CF that is on average ~ 300 sp/s, a significantly higher rate than found in ANFs (~ 100 sp/s for the last 70 % of the

response, Rhode and Smith, 1985); in comparison, OnL unit steady-state rates are often lower, sometimes < 50 sp/s. The maximum discharge rates for units with CFs below a few kHz always occur between 600 and 800 Hz and can equal the stimulus frequency, i.e., 600-800 sp/s. All PLn units do not always have a high degree of phase locking at low frequencies, at least for stimulus levels less than 90 dB SPL (e.g., Fig. 8A). In fact, some units have no response to low frequency stimuli for intensities less than 90 dB SPL. The degree of receptive field asymmetry varied with the PSTH pattern. This may occur because GBCs that have a PL response often have a higher sustained rate than OnL units at CF, resulting in less asymmetry in the receptive field. Also PL units do not entrain as well as PLn/OnLs and some do not entrain at all, so that their maximum discharge rate is less than that of PLn/OnLs.

Some of the variability in both receptive fields and PSTHs is likely due to the amount of inhibitory input. It may be that inhibition is responsible for the low sustained discharge rates in some GBCs. Generally, the portion of the receptive field above the excitatory region was not explored because inhibition is not apparent in low-SR units and it was not the focus of this study. Therefore, the frequency of occurrence of a high-frequency inhibitory effect is unknown, as is whether inhibitory inputs play a role over the entire frequency range. However, inhibition was reported in nearly all fibers with a PLn response that were recorded in the trapezoid body (Spirou *et al*, 1990).

3D view of globular/bushy cells

The CN was always cut in the coronal plane and 3D data were captured using the NeuroLucida system so that the CN could be rotated in any direction to obtain a horizontal or sagittal view. Three orthogonal views of a GBC classified as PLn are shown in Figure 4. GBC dendritic fields are usually limited to a 50-100 μ m region around the soma. The number of primary dendrites ranges between one and five, with one being the norm. Axon diameters near the cell body were on the order of 3-4 μ m (note shrinkage due to fixation was not determined but is likely less than 10%). The axon diameters including myelin cannot be determined with this labeling method but have been reported to be 8-9 μ m (Smith *et al*, 1991). With appropriate magnification and labeling, swellings in the dendrites are clearly visible and have been shown with electron microscopic techniques to contain large mitochondria (Cant and Morest, 1979). A three-orthogonal view reconstruction of a GBC with a low CF is shown in Fig. 5, together with its receptive field and PSTH. Rotation of the original coronal drawing makes it apparent that this cell has two dendritic fields that are on opposite sides of its soma.

Intracellular recordings

It was relatively rare to obtain high quality intracellular recordings from the *in vivo* preparation as entry into the cell was often followed by a rapid decline in the membrane potential. Often the recording appeared to be from the axon near the soma, as the spike amplitude was relatively large and the EPSPs were relatively small. Axonal recording was confirmed when a retraction bulb, a swelling in the axon, was observed, because this indicates the injection site. When the soma was penetrated with the electrode, usually no spike was visible because of the relatively low somatic membrane resistance, which attenuates the spike propagated back from the initial segment of the axon (Oertel, 1983; Rhode *et al*, 1983). In somatic recordings from bushy cells, often EPSPs cannot be unambiguously distinguished from spikes.

An intracellular FM sweep response illustrates the superposition of EPSPs and spike amplitudes as the stimulus frequency was varied from a low to high value (Fig. 6A). At low frequencies, EPSPs are more likely to sum, which results in a systematic decrease in EPSP amplitude with increasing frequency (Fig. 6B). The width of the early events is around 4 ms while what appears to be spikes after 0.3 s have a 1 ms width. The early event indicated by arrow 1 in Fig. 6B has several discontinuities suggesting multiple EPSPs were summed and are

indicated by the solid filled circles on the falling side of the waveform. The waveform indicated by arrow2 is much narrower and occurs $\sim 180^\circ$ out of phase with the prior event. It also has an inflection (plateau or maybe noise) in the waveform indicated by the solid circle. Amplitudes of out-of-phase 'spikes' decrease as frequency is increased (arrow 3). With increasing stimulus frequency, the out-of-phase spikes merge with the portion of the response that is in-phase and eventually cease to occur. Arrow3 indicates this reduced response where there are several EPSPs superimposing without spike generation. This out-of-phase firing may underlie the decrease in VS at low frequencies in GBCs and likely is due to 'peak splitting', i.e. discharges out-of-phase with the low-intensity discharge phase. Peak-splitting is known to occur in the AN (Ruggero and Rich, 1983). It is likely that the near simultaneous occurrence of EPSPs at low frequencies results in large phase-locked intracellular potentials that are somewhat spread out over a stimulus cycle even at 100 Hz. The probability of superposition of EPSPs from ANFs decreases with increasing frequency as a result of a decrease in their phase locking (Johnson, 1974).

A portion of the individual intracellular records for intensity/frequency combinations from a complete response area illustrates that for very low stimulus frequencies GBCs can fire twice per cycle (Fig. 7, 200 Hz top panel). This is analogous to the two events per stimulus cycle seen in the low frequency portion of the sweep response (Fig. 6). As the stimulus frequency is increased, the neuron no longer responds with one spike per cycle but rather skips stimulus cycles before firing again.

Phase locking in GBCs

At 400 and 800 Hz, there is one event per cycle at an exact phase (Fig. 7). As the stimulus frequency is increased, e.g. to 1600Hz, the unit can no longer follow each cycle though a stimulus synchronous EPSP is present (see arrow). Phase-locked EPSPs are seen for stimulus frequencies up to 2200 Hz in Figure 7 (note the arrow on the 2200Hz panel where the small ripples in the waveform are at the stimulus frequency). Two kilohertz is about the highest frequency where significant phase locking activity is observed in PLn units (Joris *et al*, 1994a).

Figure 8A illustrates the range of VS observed for two PLn cells. High VS at three intensities (70, 80 and 90 dB SPL) is shown in solid lines where VS is nearly 1.0, i.e. perfect phase locking (Fig. 8A). This is in agreement with other GBC studies indicating these neurons have excellent phase locking properties (e.g., Smith *et al*, 1991; Joris *et al*, 1994a). In contrast, VS for a PLn unit indicated by the three dashed lines is significantly lower, more akin to the VS of ANFs illustrating that not all of these units have exceptional phase locking.

In comparison, the range of VS's for ANFs with similar CF's but different spontaneous rates illustrates that low spontaneously active ANFs (LS-ANFs, LS rate < 1 sp/s, Fig 8B, solid lines) have significantly higher VS's than high spontaneously active ANFs (HS-ANFs, HS rate > 18 sp/s, Fig. 8B, dashed lines, two fibers). This difference in VS as a function of spontaneous rate was first noted by Johnson (1980).

A composite of the VS functions for PLn & OnL units in this study with CFs < 1 k Hz at 80 dB SPL is shown in Fig. 8C and can be compared to the VS functions for ANFs with CFs < 1 kHz in Fig. 8D. The VSs of PLn and OnL units are often nearly 1.0 for frequencies < 500 Hz while ANFs rarely have a VS > 0.8 regardless of their spontaneous rate. VSs are highest for both PLn and OnL units with CFs between 1 and 2.5 kHz (Fig. 8E). In this CF range, ANFs have VSs that occasionally reach 0.95 at low frequencies (Fig. 8F). PLn/OnL units are seen to have VSs as high as 0.99, though their range of VSs includes that of ANFs. Both ANFs and PLn/OnL units have the highest VSs for their low-SR members. For CFs > 6 kHz, PLn/OnLs and ANFs all have lower VS than in the lower CF range (Fig. 8G and 8H). The spread of VSs

is similar in this range for both ANFs and units with the PLn/OnL pattern. VS for PLn/OnL units decreases faster as a function of increasing frequency than VS does for ANFs. Additionally, their VS is rarely significant (Rayleigh coefficient < 0.001) for stimulus frequencies above 2 kHz (Fig. 8G) while the VS of ANFs is often significant for frequencies > 2.5 kHz.

Other aspects of VS convey GBCs ability to preserve temporal information for higher auditory centers. For example, irrespective of the CFs shown in Fig. 8, the maximum value of VS in PLn/ OnL units far exceeds the VS which would exactly encode a sinusoidal stimulus (VS = 0.754 for a half-wave rectified sinusoid). This is especially true for units with CFs between 1 and 2.5 kHz, where VSs up to 0.99 are frequently found. Fast channel kinetics of GBCs undoubtedly play a role in achieving this result. Significant overlap between the distributions of VS versus frequency found for GBCs and ANFs (Fig. 8E and 8F) could be interpreted to indicate that single ANFs may be sufficient to discharge the unit. There may also be a preference for inputs from low spontaneous ANFs either in number of synapses or their postsynaptic strength, at least in some GBCs.

PLn units also phase lock well to the envelope of AM signals as shown for PLn neurons in Figure 9. Here, the magnitude of temporal modulation transfer functions (TMTF, VS *versus* modulation frequency) indicate that there is enhanced phase locking to the modulating frequency. That is, VSs as high as 0.95 (e.g. Fig. 9D) are significantly higher than one expects from exact encoding of a 100% modulated AM signal, which would produce a VS of 0.5 if the AM envelope were exactly duplicated by a unit's discharges. Most TMTFs of PLn units are bandpass in shape, which differs from the low pass TMTFs of ANFs (Joris and Yin, 1992). A second indication of selective feature enhancement is that VS decreases less with increasing intensity (indicated by increasing line thickness) than seen in ANFs (Rhode and Greenberg, 1994). This is an example of improved coding of temporal signal features in the CN unit population that may play an important role in signal processing in higher auditory centers.

Discussion

Variety of PSTH patterns

Osen (1969) might be described as a “lumper” in terms of her categorization of morphological cell types in the CN, as she proposed nine categories versus the 55 that Lorente de N6 (1981) described. It is likely that some of the de N6 categories were variations within a single category. Later studies of response physiology showed that a single morphological unit type can exhibit multiple temporal response patterns. This was best shown in the dorsal CN where fusiform cells exhibit a variety of PSTH patterns (descriptively labeled pauser, buildup and chopper) that depend upon the intracellular potential (Rhode and Smith, 1986b; Manis, 1990).

In contrast, it is likely that the range of response patterns observed in GBCs is a result of the superposition of ANF afferent EPSPs, whose number and spontaneous rates (SR) may vary from cell to cell. Modified end bulbs vary greatly in size, with the high-SR fibers having the largest and most numerous endings on GBCs. Liberman (1993) found an average convergence of 19.2, 2.9 and 2.6 modified end-bulbs from high-, medium-, and low-SR fibers respectively, and indicated higher convergence estimates would result if all ANF endings were included. Using serial-section electron microscopy to reconstruct the innervation of GBCs, Spirou (Spirou et al., 2005) found that between 9 and 69 ANFs make contact with GBCs, with 23 being the average as taken from 12 GBCs reconstructions. Simultaneous arrival of a large number of EPSPs presumably results in a probability of near one that GBCs will discharge at stimulus onset. For low frequency stimuli, it is likely that many EPSPs simultaneously arrive at a particular stimulus phase, resulting in a high degree of phase locking (Fig. 6, 7, 8).

It has been suggested that GBCs could fire with relatively few AN inputs (Rhode and Smith, 1986a). This was based on a scatter diagram comparing the spontaneous and maximum discharge rates of a relatively small number of PLn units, where clusters of points suggested that there could be 1-3 high-spon ANFs contacting the cell and that each ANF generated EPSPs which were above threshold (see discussion in Spirou *et al*, 1990). A comparison of the scatter diagram of maximum discharge rate *versus* SR for the population of GBCs in the present report showed that there are clusters of points, suggesting that individual endbulbs can discharge the postsynaptic element, i.e., all endbulbs are not created equal in terms of synaptic strength. This does not mean there are only a few endbulbs contacting the cell, only that there are a few synaptic events capable of individually causing the GBC to discharge.

Cellular modeling studies have usually begun with a “perfectly spherical soma” and ignored the dendritic input where there are AN contacts (Spirou *et al*, 2005). Yet by varying the number and efficacy of each input on the soma alone, the range of PSTH patterns associated with GBCs could be duplicated, from PLn to OnL (Spirou *et al*, 1990; Spirou *et al*, 2005; Rothman *et al*, 1993). Though Spirou *et al* found that the inhibitory inputs covered a relatively small portion of the GBC soma (~5%), some inhibitory inputs were necessary in the models to duplicate the entire range of PSTH patterns (Rothman *et al*, 1993). Even though inhibitory inputs on the soma constitute only a few per cent of the total synapses, in these studies there are a few instances of upper inhibitory sidebands in GBC receptive fields, indicating that this small number of inhibitory inputs can have significant influence on unit behavior, and possibly even at CF. Inhibitory sidebands were generally found both above and below CF (Spirou *et al*, 1990).

There is a discrepancy between the present PSTHs and those of Smith *et al* (1991), who showed that nearly all of their response patterns were OnL with very low sustained rates, even as low as a few spikes/s. Their PSTHs also showed significant adaptation over the 25 ms duration of their stimulus. In contrast, the average sustained CF discharge rate of PLn's in the present study is over 200 spikes/s at CF, with instances up to 350 spikes/s and rate adaptation often being absent (Fig. 1). One possible explanation is that the recordings here were from damaged cells. This is unlikely, or at least not the norm, as many recordings were axonal or extracellular. Another possibility is that there is a sampling bias when recording from axons in the trapezoid body, because the largest axons undoubtedly are the easiest to isolate or that there are spike failures at branch points along the path to the trapezoid body.

The principal role of GBCs in the lateralization of high frequency sounds is to provide the inhibitory/suppressive contralateral input to the lateral superior olive (LSO) via the MNTB. The inhibitory input to the LSO suppresses LSO activity that originates from the ipsi-lateral input of spherical/bushy cells, the major excitatory input to the LSO thereby creating a neural representation or code for the interaural intensity differences used to localize high-frequency sounds (Cant and Casseday, 1986).

Phase locking in GBCs

Based on recordings from their axons in the trapezoid body, the phase locking capabilities of GBCs has been described as extraordinary (Smith *et al*, 1991; Joris *et al*, 1994a). Results reported here comparing GBC and ANF phase locking indicate GBCs phase lock significantly better than their ANF inputs in the frequency range < 1 kHz. That is, these results essentially agree with earlier reports as VSs up to 0.99 (near perfect entrainment) occur in GBCs at low frequencies when GBC CFs are between 1 and 2.5 kHz, while such high VSs are never observed in ANFs with similar CFs. In the higher CF region (> 6000 Hz), data demonstrate that some ANFs have at least as good phase locking as PLn/OnL units (Fig. 8E & 8H). It was reported that PLn units with CF > 3 kHz have nearly perfect entrainment to low frequency signals, i.e., in the tail region of their receptive fields (Joris *et al*, 1994b). Here, VS *versus* frequency

functions are typically < 0.9 (Fig. 8G). This difference may result from their reporting VSs mostly at 500 Hz. Another factor may be Joris *et al*'s use of a peak-picking device to determine the time at which the maximum of a spike occurs, rather than an oscilloscope level trigger which was used in the present study. Peak-picker circuits are influenced by the presence of stimulus related potentials that would result in higher VSs. However, I was assured that no such potentials were present during the recordings (P. Smith, per. comm.).

There is some difference between studies in the magnitude of GBC phase locking improvement depending on CF, and low-SR ANFs shown here in Figure 8 can have VSs that reached 0.95, values somewhat higher than previously reported. However, there is a general agreement that low-SR ANFs have higher VSs than high-SR ANFs (e.g., Joris *et al*, 1994a; Johnson, 1980), and the present results indicate that there is a significant CF factor. Spirou *et al*'s (2005) GBC model simulations found generally there was no improvement in phase locking for GBCs relative to that of ANFs. Liberman (1993) reported that there are many more synaptic contacts from high-SR ANFs on GBCs than from low-SR fibers. If the synaptic contacts of ANFs with different spontaneous rates are of equal strength, then one would expect that the VS of GBCs would be closer to that of the high-SR ANFs than low-SR ANFs. This is not the case in the 1 – 2.5 kHz CF region (Fig. 8E and 8F), therefore, an alternate explanation is required. Information from studies of octopus cells, where low-frequency phase locking more extraordinary than GBCs was demonstrated (Oertel *et al*, 2000), suggest that fast membrane time constants must play a major role in determining a neuron's phase locking capabilities. In octopus cells, extraordinary phase locking is a result of membrane properties that not only require coincidence of the ANF inputs (EPSPs) but a fast rate of change of the depolarizing event to discharge the neuron (McGinley and Oertel, 2006). GBCs have somewhat similar membrane properties (Manis and Marx, 1991; Oertel, 1983; Rothman and Manis, 2003a) to octopus cells in that they also require a rapid rate of rise in the intracellular potential that could enhance phase locking relative to that of the high-SR ANFs.

Joris (1996) reported significant phase locking to AM signals in the LSO, indicating the preservation of low-frequency envelope information at this level of the brainstem. The bandpass TMTFs reported here demonstrate that GBCs can be reliable encoders of AM. Results also show GBCs can have significant VSs at high intensities, even better than ANFs, where VS decreases at high intensities (cf. Rhode and Greenberg, 1994). Overall, these data suggest envelope coding is preserved by the MNTB neurons that project to LSO and may be used in localization of sounds.

Projections of GBCs

Injecting GBCs in the CN often results in incomplete labeling of their axonal projections. By injecting the axons of GBCs in the trapezoid body, Smith *et al* (1991) were able to describe their projections in the brainstem in considerable detail. Besides terminating in one or two calyces in the MNTB, GBCs have as many as four additional ipsilateral and three more contralateral targets. These targets include several periolivary nuclei that likely provide feedback control to several centers, including the cochlea. It is a speculation that the GBC projections provide a measure of the stimulus level and that these neuronal subsystems are involved in some descending regulation of auditory system activity which could function as a protective mechanism for the cochlea.

Conclusions

The fast membrane kinetics of globular-bushy cells located in the nerve root area of the cochlear nucleus results in their firing in response to coincident inputs. Their range of PSTH response patterns to repeated short tones results from a wide range of the number and spontaneous rates of their ANF inputs. Estimates of ANF convergence on GBCs range from a few to over 60

inputs. Additional inputs to the dendritic portion of the GBCs are restricted to a region close to the soma by virtue of the short extent of their dendrites. Therefore, ANF afferents to nearby GBCs could provide a sample of their input to another GBC *via* its dendrites. It is also possible there are inputs from as yet other unidentified sources. GBC axons exit the CN without contacting any local targets (Smith and Rhode, 1987). With excellent low frequency phase locking and representation of the signal envelope, GBCs could be a key element in encoding both temporal and intensity aspects of acoustic signals. Because GBCs function in part as efficient input coincidence detectors, they emphasize changes in the acoustic signal, and thereby alert the system to the presence of any new information. The combination of excellent low-frequency phase locking, envelope encoding (AM phase locking) and signal change encoding (onset emphasis), suggests that GBCs play a role in aspects of signal identification beyond source localization. This is likely given that the GBCs provide an important input to the VNLL, a monaural processing nucleus, via their input to the MNTB which in turn projects to the VNLL. GBCs could be useful in processing species specific signals in general.

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Figure 1.

PSTHs for 9 GBCs with a range of CFs. A principal feature is a 0.5 -2 ms pause in activity after the initial spike that is likely the result of the neuron's refractory period. A pause only occurs when there is an onset spike for nearly 100% of the trials. Unit CF and spontaneous rate (SR in spikes/second) are indicated in each panel. Binwidth is 100 μ s.

Figure 2.

Responses of a PLn unit at four intensities, 30 to 90 dB SPL in 20 dB steps. Threshold = 18 dB SPL and CF = 10165 Hz. 50 ms tones of which only the initial 26 ms is shown. The firing rate remained constant beyond 20 ms at each intensity level.

Figure 3.

Receptive field maps for six units that exhibited a P_Ln/OnL response pattern for a range of CFs, as indicated in each panel. All the data but that in panel D were obtained from labeled GBCs. The neuron from which panel D was obtained was not successfully labeled after recording intracellularly for an extended period. A higher discharge rate at low frequencies results from the tendency of GBCs to try to discharge on every stimulus cycle at a precise phase, to low frequency stimuli (E). Panels C and F illustrate inhibition of spontaneous activity in the frequency region above CF, where the curves fall below the baseline of the receptive field (indicated by the triangular symbols). In each panel, increasing line thickness indicates increasing intensity, which varies from 0 to 90 dB SPL in 10 dB increments. Solid and dashed lines are alternated for clarity. The 30 and 90 dB SPL curves are labeled in panel F.

Figure 4.

Three orthogonal views of a GBC with a single dendrite and the axon is shown in red. For the illustration at the top, the 3D morphology was obtained from tracings of coronal sections. The bottom two orthogonal views were computer generated using Neurolucida (MicroBrightField). Insets: A. receptive field for the cell where the linewidth increases with stimulus intensity that varies from 0 to 90 dB SPL in 10 dB steps. B. PSTH at CF = 9039 Hz and 60 dB SPL.

Figure 5.

Three orthogonal views of a GBC with two primary dendrites. Axon is shown in red. The 3D morphology was obtained from tracings of coronal sections (top). The other orthogonal views were computer generated. Insets: A. receptive field where the linewidth increases with stimulus intensity that varies from 0 to 90 dB SPL in 10 dB steps. B. PSTH at CF = 875 Hz and 60 dB SPL.

Figure 6.

A. Intracellular FM sweep recording from a GBC where the sinusoid was varied between 100 and 1000Hz. S indicates one of the axonal discharges propagated from the axonal initial segment to the soma. B. Expanded time scale for the first 75 ms of data in A emphasizes the large synchronous potentials at low frequencies. Note that at the lowest frequencies there can be two EPSPs per stimulus cycle where the second EPSP is narrower than the first (arrow 1 and arrow 2) and its amplitude decreases systematically with increasing frequency (arrow 3).

Figure 7.

Intracellular records for a GBC with an OnL PSTH illustrating a stimulus synchronous membrane potential in response to five stimulus frequencies up to 2200 Hz (arrow in the bottom panel indicates the 2200 Hz intracellular potential). Note that the cell discharges on both the positive and negative phase of the 200 Hz stimulus (top panel); compare with the 400 Hz response in the second row. For this neuron, CF= 4300 Hz, Th = 10 dB SPL and SR = 16.5 spikes/s.

Figure 8.

A. VS *versus* frequency at three intensities (70, 80 and 90 dB SPL) for two PLn neurons with CF = 2400 Hz (solid line) and CF = 1600 Hz (dashed line), respectively. Increasing line weight corresponds to increasing intensity in all panels. B. VS for three ANFs, each with a CF $\sim$ 1900Hz, illustrated by dashed (SR = 90 sp/s), dash-dot (SR = 79 sp/s) and solid (SR = 1 sp/s) lines respectively, CFs = $\sim$ 1900 Hz. The VSs in all remaining panels were taken at 80 dB SPL. There was little variation in VS as a function of intensity for SPLs sufficiently above threshold. C. VS *versus* frequency for a 3 PLn/OnL units with CFs < 1 kHz taken at 80 dB SPL. D. VS for 16 ANFs for the same level and frequency range as in C. E. VS for 7 PLn/OnL units with CFs between 1 and 2.5 kHz. F. VS for 21 ANFs for the same conditions as in E. G. VS for 6 PLn/OnL units with CFs > 6 kHz. H. VS for 6 ANFs for the same conditions as in G. In all panels: solid lines indicate SR (< 18 Spikes/s) and dashed lines indicate high-SR (> 18 spikes/s). Data from PL units judged to be from units in the NRA were not included here, though inclusion did not appear to significantly alter the results. ANF data are from Rhode and Smith's (1985) cat study.

Figure 9.

TMTFs shown at three different intensity levels for six PLn units with the CFs 700 to 7000 Hz. Modulation depth was 100%. AM intensity was 30 to 70 dB SPL in 20 dB steps as indicated by increasing line thickness. Signal duration was 100 ms and was repeated every 200 ms, 20 times. In each instance, the carrier frequency was set to CF except for the unit in A, where it was set slightly higher at 1000 Hz.