THE anteroventral cochlear nucleus (AVCN), the first centre of the central auditory pathway, contains globular bushy cells, which are unique in their ability to produce fast excitatory post-synaptic potentials (EPSPs). Using *in vivo* intracellular recordings in the rat AVCN we examined these fast EPSPs in relation to temporal coding. At frequencies up to 2.5 kHz, EPSPs were evoked on successive sine waves of the stimulus with EPSP summation limited. This one-to-one relationship between the EPSPs and the sound wave period was present at higher frequencies and over a greater intensity range than for action potentials. These results suggest that temporal coding is possible in globular bushy neurones by their ability to extract temporal information through fast processing of convergent presynaptic input.

Key words: Auditory neurophysiology; Bushy cells; Cochlear nucleus; Intracellular recording; Temporal coding

Intracellular responses of the rat cochlear nucleus to sound and its role in temporal coding

Antonio G. Paolini,^{1,CA}
Graeme M. Clark^{1,2} and
Anthony N. Burkitt²

¹The Human Communication Research Centre, Department of Otolaryngology, University of Melbourne, Royal Victoria Eye and Ear Hospital, 32 Gisborne Street, East Melbourne 3002; ²Cooperative Research Centre for Cochlear Implant, Speech and Hearing Research, Melbourne, Australia

CA Corresponding Author

Introduction

Temporal coding of sound is manifested by the intervals between neural responses being multiples of the period of the sound wave.^{1–5} Temporal processing of acoustic information was thought to rely on at least one or more auditory nerve fibres within a group producing an action potential on a cycle of the input stimulus.⁶ However, although individual auditory neurones fire in phase with each sine wave,^{3,4,7} they usually do not respond to each cycle above ~0.6 kHz.⁸ Nevertheless, at frequencies below ~2.0–4.0 kHz there are populations of intervals that are multiples greater than one of the period of the sound wave.⁹

Recent extracellular evidence has shown that first-order central auditory neurones in the anteroventral cochlear nucleus (AVCN) are able to phase-lock in a more precise manner and show higher entrainment capabilities than auditory nerve fibres.¹⁰ The AVCN contains two predominant cell types, the stellate and bushy cell, both of which receive monosynaptic input from auditory nerve fibres emanating from sensory neurones in the cochlea. However, bushy cells are able to phase lock with greater synchrony and over a wider range than stellate neurones,¹¹ suggesting that they may play a role in temporal coding.

An important aspect of temporal coding is that the sum of converging inputs on dendritic and somatic

elements of the neural processor maintains temporal information. It has been postulated¹² that the brain codes temporal information in a population of neurones when APs along converging inputs are coincident, and arrive within a certain time window at the nerve cell which subsequently produces an AP. Temporal coincidence of presynaptic excitation may be required to cause an AP discharge in these bushy cells. However, how temporal information is maintained by convergent input is not clear.

In vivo intracellular recordings from bushy cells described as globular have demonstrated large, fast synaptic potentials in addition to a larger presumed AP to acoustic stimulation.¹³ Smith and Rhode¹³ suggest that these fast EPSPs arise from endbulbs of Held¹⁴ and possibly other smaller auditory nerve terminals. Convergence of auditory nerve fibres and the ability of globular bushy cells to produce fast EPSPs require further intracellular investigation.

In the present study, intracellular recordings were made from identified globular bushy cells in the AVCN in order to provide a neural basis for temporal coding. Our aim was to determine how well EPSPs in globular bushy neurones preserve temporal information over a wide range of frequencies and intensities compared to that of APs. As a result of this study we found a good correlation between the fast EPSPs and the period of the sound wave with temporal following of EPSPs best explained by the

presence of convergent input onto these globular bushy neurones.

Materials and Methods

Preparation: All experiments were performed on 11 male hooded rats anaesthetized with urethane in water (1.3 g/kg, i.p.), and breathing spontaneously. Supplemental doses were administered if a strong corneal or paw reflex was observed at any time during the experiment. All efforts were made to prevent any animal suffering in accordance with the Royal Victorian Eye and Ear Hospital animal ethics guidelines (Grant 95037).

After a craniotomy the cerebellum was aspirated on one side to expose the cochlear nucleus enabling intracellular recording electrodes to be inserted into the anteroventral cochlear nucleus under visual control. Core body temperature was maintained at $\sim 37^{\circ}\text{C}$ with a DC homeothermic blanket.

Recording: Microelectrodes made from thin-walled (1.0 mm o.d.) quartz glass (Sutter), were filled with 1 M potassium acetate (70–80 M Ω) or in some cases 4% Neurobiotin in 1 M potassium acetate as described by Paolini and McKenzie¹⁵ and advanced through the anteroventral cochlear nucleus. When stable cell impalements were obtained, acoustic stimuli were delivered to the animal. Recordings were possible for up to 40 min. Globular bushy cells were distinguished by their intracellular responses, which typically showed fast synaptic potentials (EPSPs) together with larger APs.¹³ A MacLab 4S data acquisition system (AD Instruments) was used to store electrophysiological traces at a bandwidth of 20 or 40 kHz. Acoustic stimuli were produced by a Beyer DT48 transducer which was positioned at the end of a hollow ear bar, and controlled using a PDP-11/34 computer. The acoustic system was calibrated using a Bruel & Kjaer (B&K) measuring amplifier (type 2606), and a second B&K 1/2 inch condenser microphone coupled to a small probe tube positioned within the ear bar tube approximately 3 mm from the tympanic membrane to enable acoustic input to be measured in dB sound pressure level (SPL).

Once impaled, the neurone's characteristic frequency (CF) and acoustic input-output functions to frequencies at or close to CF were determined. The CF was calculated from an acoustic threshold tuning curve which was constructed on-line, as described in detail by Liberman.¹⁶ Artefact as a cause of the fast synaptic potentials (EPSPs) has been excluded as they were not recorded at the onset of the stimuli (Fig. 1) or in other cell types at low frequencies (our unpublished observations).

FIG. 1. Neural response to acoustic stimulation in the anteroventral cochlear nucleus (AVCN). (A) This neurone showed fast synaptic potentials, typical of globular bushy cells,¹³ together with larger presumed APs to tones (left; horizontal bar indicates onset and cessation of stimulus presented for 55 ms). The response to a 7.3 kHz tone presented at 55 dB SPL is shown. This cell, located in the most lateral extent of the AVCN (insert), was identified as a globular bushy cell with its single primary dendrite branching profusely forming a tuft arrangement. (B) Intracellular response from globular bushy cell type in the rat anteroventral cochlear nucleus: (top) excitatory postsynaptic (EPSPs) or fast synaptic potentials and action potentials (APs) phase-locked to the stimulus; (bottom) a pure acoustic tone of 1.0 kHz at 90 dB SPL. The EPSPs but not the APs usually occur on successive sine waves.

Acoustic input-output functions were constructed initially at the CF. The tone was delivered in 5 dB steps in a sequential manner from sub-threshold to saturation intensity. With each increase in intensity, up to 50 repetitions of the stimulus were presented. Between each presentation the neurone was allowed to rest for a period of 1.5 s. The stimulus typically consisted of a 50 ms burst with a 5 ms rise and fall time and a 5 Hz repetition frequency.

Two cells were filled with Neurobiotin by passing approximately 1.1 nA of depolarizing current pulses (500 ms duration, 1 Hz repetition) through the micropipette for 5–10 min.

Histology: The cochlear nucleus was examined for Neurobiotin-filled cells in two animals. After an anaesthetic overdose the rats were perfused transcardially with 10% formalin containing 30% sucrose. They were decapitated and the head dissected free and preserved in sucrose-formalin solution. After 2 days the brains were removed and sectioned at

FIG. 2. Globular bushy cell response to acoustic stimulation. (A) This neurone had a characteristic frequency (CF) of 2.5 kHz and a threshold of ~ 10 dB SPL. (B) The acoustic input-output (IO) function for action potentials (APs) showed a non-monotonic response to stimulation at 1.0, 1.5 and 2.5 kHz over the tested dB range. The response curve was similar across these frequencies with a shift to the right and a narrowing of the response area with decreasing frequency. (C) Acoustic IO function for EPSPs at 1.0 and 1.5 kHz showing a monotonic rise. (D-F) Intracellular response (top) with expanded time base (middle) and period histogram (bottom) for tones presented at 1.0 kHz (80 dB SPL), 1.5 kHz (70 dB) and 2.5 kHz (40 dB SPL). Horizontal line under top panels indicates onset and cessation of stimulus. EPSPs were seen on successive cycles of the stimulus (middle) with APs showing phase-locked activity (bottom).

FIG. 3. EPSP inter-peak (left) and AP inter-spike (right) interval histograms for a globular bushy cell presented with a tone of 1.0 kHz over increasing stimulus intensities from 75 to 105 dB SPL. Histograms were constructed for 12 stimulus presentations at 75 dB SPL, and for 20 presentations at all other intensities (Figs 3,4).

120 μ m parasagittally on a freezing microtome. Brain sections were processed using avidin-horseradish peroxidase (HRP) and intensified DAB.¹⁵

Data analysis: The data were analysed off-line by a peak detection program. An algorithm was used to identify spikes and peaks within a time window which varied from 0.15 to 0.5 ms, depending on the frequency. After establishing the times at which these spikes and peaks occurred, AP inter-spike and EPSP inter-peak interval histograms were constructed which showed the probability of intervals occurring.

Results

Intracellular recordings: Stable intracellular recordings were obtained from 17 neurones in the AVCN. They responded with small AP amplitudes and fast synaptic potentials, two of which were identified morphologically as globular bushy cells (Fig. 1A).

Stable neurones were held for at least 3 min and up to 40 min, and had a mean ($\pm$ s.e.) resting membrane potential of -53.1 ± 2.1 mV with an AP amplitude (with corresponding ranges) of 26.4 ± 1.4 mV (20–40 mV). The CF ranged from 0.9 to 37.0 kHz with a mean threshold intensity at CF of 37.8 ± 4.9 dB SPL (0–80 dB SPL).

Responses to stimulation: Of the 17 neurones, 11 were spontaneously active. With an acoustic input at CF these neurones responded in a primary-like manner¹⁷ with AP firing probability increasing during the initial stages of the stimulus before declining to a steady discharge level. In five of the 15 cells tested there was a short interval during which no APs fired after the initial spike discharge, before firing returned to a steady state for the remaining stimulus duration (primary-like with notch response). The intracellular response to tones also demonstrated smaller fast EPSPs which could occur < 1 ms after each other. These fast EPSPs varied in amplitude, had a constant short duration of less than a millisecond (Figs 1,2)

FIG. 4. EPSP inter-peak (left) and AP inter-spike (right) interval histograms for a globular bushy cell presented with a tone of 2.5 kHz over increasing stimulus intensities from 35 to 110 dB SPL.

and were present predominantly during the presentation of the tone, although all cells exhibited spontaneous EPSPs. No spike or EPSP adaptation was seen within each intensity presentation.

In 10 cells the EPSPs were predominantly phase-locked and present at successive cycles of stimulus for frequencies ≤ 2.5 kHz (Figs 2–4). These EPSPs also increased in amplitude with increasing sound intensity (Fig. 5A,B). Apart from temporal jitter (Figs 3,4), the correspondence between the peak of the EPSPs and the waveform of the stimulus did not vary systematically during the tone presentation. The extent to which EPSPs occurred on successive sine waves became greater the lower the frequency (Fig. 2). An example of how much better EPSPs are than APs in following successive sine waves can be seen in neurones presented with tones of 1.0 kHz, as shown in Figs 1B and 2D.

The populations of time intervals between APs and EPSP peaks were compared across frequencies, and over the intensity range from threshold to a maximum stimulus of 110 dB SPL. Histograms

of intervals for a response at 1.0 kHz are shown in Fig. 3. There were two peaks for the EPSPs, the larger being the same as the period of the sound wave over a 30 dB SPL range. With APs, there were a larger number of peaks that were multiples of the period, and no predominant one, except at the highest intensity. The mean firing rate for APs fell at the highest intensities (non-monotonic), but this was not seen for the EPSPs.

As the frequency increased, peaks in EPSP inter-peak histograms occurring at multiples of the stimulus phase became less distinct. The AP inter-spike and EPSP inter-peak histograms from a cell in response to 2.5 kHz are shown in Fig. 4. From this it can be seen that as with the response to 1.0 kHz (Fig. 3) the predominant interval for the EPSPs was the same as the period of the sound wave, although there was a trend for it to merge with smaller subsequent peaks. Furthermore, the predominant peak occurred over a 75 dB SPL intensity range.

In seven cells the ability to follow successive cycles of the stimulus was no longer apparent at

frequencies of ≥ 2.7 kHz, with summation of depolarizing events being more common. Increased sound intensity in these neurones resulted in an increase in summation of depolarizing potentials (Fig. 5C,D).

Acoustic input-output AP functions had a mean dynamic range of 22.9 ± 2.6 dB SPL in 12 cells tested. Among these 12 cells tested, a rise in discharge rate with increasing sound intensity (monotonic rise) was observed in four with the remaining eight showing an initial increase followed by a drop in discharge rate (non-monotonic rise). Although AP firing rate decreased at higher intensities for non-monotonic and plateaued for monotonic neurones, this was not the case for the rate of EPSPs in low frequency neurones which showed monotonic relationships with intensity with a continued rise in rate over the intensity range tested (from 40 to 115 dB SPL; Fig. 2C). It was not possible to examine individual EPSPs at frequencies > 2.7 kHz as their summation prevented identification of distinct peaks.

Discussion

In this investigation, temporal information appears to be preserved by globular bushy cells at frequencies up to 2.5 kHz by the interval between fast excitatory post-synaptic events. An important feature of these globular bushy cells is their ability to process information quickly. The presence of these fast EPSPs in these neurones and the ability to produce EPSPs on each cycle of the stimulus is important for temporal coding. Intracellular recordings from globular bushy cells have shown that these fast synaptic potentials occur within 1 ms of one another. The fast nature of this synaptic response allows EPSPs to follow successive sine waves and, as proposed by Smith and Rhode,¹³ precise coincidence of two or more of these fast EPSPs would be important for cell activation.

The fast nature of the synaptic response in globular bushy cells may be due to their biophysical properties. Support for this ability to produce fast EPSPs has been provided by Manis and Marx,¹⁸ who showed that bushy cells had short membrane time constants. *In vitro* studies by Oertel¹⁹ have shown bushy cells to have highly non-linear current-voltage functions. Rhode and Greenberg¹⁷ suggest that this non-linearity is due to ion channels opening and thereby lowering the membrane resistance. This would shorten the membrane time constant and effectively maintain the membrane voltage near resting level so that the cell can respond to a second input rapidly.

The fast nature of this synaptic response may also limit summation of these EPSPs suggesting that these neurones, even though they receive multiple synapses, are able to process sensory input on a

temporal basis. In neurones with larger dendritic trees, multiple synaptic contracts and larger (more typical) membrane time constants the summation would tend to reduce temporal information. Their biophysical properties and the correspondence between EPSPs and the period of the sound wave, provides globular bushy cells with the information needed to extract temporal code. The mechanisms by which these neurones encode this temporal information may rely on intrinsic bimolecular changes. This suggests that processing of temporal information can occur in first order central auditory neurones, with the cochlear nucleus containing the elements needed for this processing in globular bushy cells.

Furthermore, the ability of EPSPs to follow successive waves of the stimulus in globular bushy cells occurred over a wide stimulus range, with the number and amplitude of EPSPs increasing with increasing stimulus intensity. Even though APs showed non-monotonic relationships between rate of firing and intensity in some neurones, the EPSP rate continued to rise with increasing intensity. Associated with this we also observed in these neurones an increase in the ability to follow successive cycles of the stimulus for EPSPs over increasing intensities. If globular bushy cells use these depolarising events to code sound frequency by extracting temporal information, they may also use the relative amplitude and number of EPSPs as a means to code intensity levels. Recently it has been proposed that EPSP amplitude differences resulting from paired pulse facilitation may provide information about recent spike occurrences which may aid in temporal discrimination.²⁰ However, as shown in this investigation the ability to code temporal information may depend not only on the relative amplitudes of EPSPs, but also on the degree of convergence and the timing of the incoming excitatory input.

A lack of adaptation of EPSPs was also noted during stimulus presentation. Unlike previous data in eighth nerve recording from goldfish,²¹ EPSP rundown was not observed in globular bushy cells during stimulus presentation. Even though more APs were recorded in the initial stages of the stimulus (primary-like response), the amplitude of the EPSPs remained constant throughout its duration. As auditory nerve fibres also fire in a primary-like fashion,²² these results suggest the presence of precise coincident auditory nerve input arrival onto globular bushy cells.

Conclusion

The data from the intracellular recordings have shown that the predominant interval between the peaks of the EPSPs remains the same as the period

FIG. 5. EPSP Phase-response ability of globular bushy neurones across frequencies and intensities. (A,B) Response at 1.0 kHz. At low stimulus spike threshold intensity (A) this neurone responded with APs followed by fast EPSPs. Increasing stimulus strength (B) resulted in an increased ability to fire EPSPs on successive cycles of the stimulus (right, expanded time base showing a 20 ms period). (C,D) Response of neurone to a 4.6 kHz tone (shown below trace). At low stimulus spike threshold intensity (C) this neurone responded with an initial spike followed by fast EPSPs. These EPSPs (right, expanded time base showing a 5 ms period) did not occur fast enough to be present on successive phases of the stimulus. With increasing stimulus intensity (D), summation of EPSPs was more prevalent (right).

of the sound wave up to a frequency of 2.5 kHz for globular bushy cells. As this relationship also occurs over a wide intensity range, this strongly supports the hypothesis that variations in membrane potential (EPSPs) are central to the temporal decoding of frequency. A one-to-one relationship with the period of the sound over a normal intensity range is not seen with APs. Although the APs are important in transmitting information as patterns of stimuli to and from nerve cells, the elements to process temporal code are present within the cochlear nucleus. The central element which provides the basis for temporal coding is the fast excitatory post-synaptic transmission of coincident information from converging auditory nerve fibres onto globular bushy cells.

References

- Rose JE, Brugge JF, Anderson DJ and Hind JE. *J Neurophysiol* 30, 769–793 (1967).
- Kiang NYS, Pfeiffer RF and Warr WB. *Ann Otol Rhinol Laryngol* 74, 2–23 (1965).
- Tasaki I. *J Neurophysiol* 17, 7–122 (1954).
- Rupert A, Moushegian G and Galambos R. *J Neurophysiol* 26, 449–465 (1963).
- Clark GM. *J Clin Physiol Pharm Res* 23, 766–776 (1996).
- Wever EG. *Theory of Hearing*. New York: Wiley, 1949.
- Rose JE, Galambos R and Hughes JR. *Bull Johns Hopkins Hosp* 104, 211–251 (1959).
- Clark GM, Irllich LS and Carter TD. *Proc Aust Neurosci Soc* 7, 115 (1996).
- Clark GM, Carter TD, Maffi CL, Shepherd RK. *Ann Otol Rhinol Laryngol* 104, 109–111 (1995).
- Joris PX, Carney LH, Smith PH and Yin TCT. *J Neurophysiol* 71, 1037–1051 (1994).
- Young ED, Shofner WP, White JA *et al.* Response properties of cochlear nucleus neurones in relationship to physiological mechanisms. In: Hassler S, ed. *Functions of the Auditory System*. New York: Wiley, 1988: 277–312.
- Carney LH. *Hear Res* 76, 31–44 (1994).
- Smith PH and Rhode WS. *J Comp Neurol* 266, 360–363 (1987).
- Brawer JR, Morest DK and Kane BC. *J Comp Neurol* 160, 491–506 (1974).
- Paolini AG and McKenzie JS. *Neurosci* 78, 229–242 (1997).
- Lieberman MC. *J Acoust Soc Am* 63, 442–455 (1978).
- Rhode W and Greenberg S. Physiology of the cochlear nuclei. In Popper A and Fay R, eds. *The Mammalian Auditory Pathway: Neurophysiology*. New York: Springer-Verlag, 1992: 94–153.
- Manis PB and Marx SO. *J Neurosci* 11, 2865–2880 (1991).
- Oertel D. *J Neurosci* 3, 2043–2053 (1983).
- Buonomano DV and Merzenich MM. *Sci* 267, 1028–1030 (1995).
- Furukawa T and Matsuura S. *J Physiol* 276, 193–209 (1978).
- Pickles J. *An Introduction to the Physiology of Hearing*. London: Academic Press, 1988.

ACKNOWLEDGEMENTS: This work was funded by the Bionic Ear Institute, Human Communication Research Centre, Hearing Research Foundation and Cooperative Research Centre for Cochlear Implant.

Received 3 August 1997;
accepted 17 August 1997

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Paolini, Antonio G.;Clark, Graeme M.;Burkitt, Anthony N.

Title:

Intracellular responses of the rat cochlear nucleus to sound and its role in temporal coding

Date:

1997

Citation:

Paolini, A. G., Clark, G. M., & Burkitt, A. N. (1997). Intracellular responses of the rat cochlear nucleus to sound and its role in temporal coding. *NeuroReport*, October, 8, 3415-3421.

Persistent Link:

<http://hdl.handle.net/11343/27563>