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Starr, A
Hellerstein, D

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DISTRIBUTION OF FREQUENCY FOLLOWING RESPONSES IN CAT COCHLEAR NUCLEUS TO SINUSOIDAL ACOUSTIC SIGNALS

ARNOLD STARR* AND DAVID HELLERSTEIN**

Department of Neurology, Stanford University, School of Medicine, Stanford, Calif. 94305 (U.S.A.)

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INTRODUCTION

If a pure tone is presented to a cat, a response having the frequency characteristics of the acoustic signal can be detected by a gross electrode within the lower stations of the auditory pathway^{1,2,4,7,13-15}. The response designated as the frequency following response (FFR) can reproduce acoustic signals from a few hundred to several thousand Hz giving it a spectral characteristic that is unique among brain potentials recorded with extracellular probes. The nature of the neural events generating the FFR is unclear, with some investigators favoring an action potential envelope theory¹⁵, others preferring a synaptic potential explanation¹³.

In an effort to shed some light upon these questions and to explore further this unusual potential, we have systematically measured the amplitude, phase, and frequency distribution of the FFR in cat cochlear nucleus.

METHODS

Successful experiments were performed on 4 cats anesthetized with sodium pentobarbital (30 mg/kg) and placed in a sound attenuating room. In two of the animals the cochlear nucleus was approached stereotactically, in the other two animals the cochlear nucleus was directly visualized through a posterior fossa craniotomy after aspirating the lateral portions of the cerebellum overlying the nucleus. Recording electrodes were 25 μ m stainless steel wires insulated to their cut end, except in the first experiment in which glass micropipettes (5 M Ω impedance at 60 $\sim$) were used. The indifferent electrode was a wound clip attached to the temporalis muscle. In all animals a stainless steel ball electrode was placed on the round window ipsilateral to the cochlear nucleus to monitor cochlear microphonic responses to the acoustic signals. Acoustic signals were presented by TDH-39 or Altec 802D speakers coupled by a plastic tube

* Present address: Department of Neurology, University of California School of Medicine, Irvine, Calif., U.S.A.

** Present address: University of California School of Medicine, La Jolla, Calif., U.S.A.

to either the hollow ear bar of the stereotaxic apparatus or to the external auditory canal. The intensity of the tone signal was adjusted to evoke a cochlear microphonic of 50–100 μV (peak to peak amplitude), and clicks were adjusted to be 20 dB above threshold for evoking a visible N1 response. Electrical activity recorded from cochlear nucleus and round window were amplified (half-power bandwidth 1.5 Hz–10 kHz), displayed on an oscilloscope, and recorded on magnetic tape (frequency response flat from DC to 5 kHz) for subsequent analysis. The earphone voltages and a pulse synchronous with the 90° phase of each sinusoid were also recorded on tape.

Our procedure was to lower the electrode through the cochlear nucleus while monitoring click evoked responses⁸. When the ventral border of the nucleus was reached, the electrode was withdrawn in 100 or 250 μm steps while recording responses to tone and click signals at each step. This procedure assures accurate spatial mapping of the nucleus³. Up to 4 tracks were made in each experiment and the electrode position marked at a defined point along several of the tracks by passage of anodal current. The animals were perfused with a solution of potassium ferricyanide and formalin and the brain stem sectioned. The location of the tracks within the cochlear nucleus could then be visualized on the slides and related to the distribution of recorded potentials.

Data on tape were averaged with a LINC computer using the synchronizing pulse from the signal generator to trigger the average. At least 16 trials were included in each average. The LINC computer was then used for computing power spectral densities, phase angles, and for preparing field potential and phase angle profiles of the spatial distributions of these results. The analysis of the averaged response began with a Fourier spectral analysis. The sine and cosine spectral components of each wave were computed using the Cooley–Tukey fast Fourier transform technique. We then confined our attention to the components of the spectrum *at the frequency of the stimulus tone*. The ratio of the sine to cosine components gave the tangent of the phase angle, from which phase angle was obtained. The root mean square power spectral density at that frequency was computed as the square root of the sum of the squares of the components. This technique has the advantage of effectively rejecting all of the signals except in a narrow range around the stimulus frequency.

Changes in capacitive coupling between electrode and tissue were not great enough to affect phase angle measurements, as verified by two control experiments. First, electrode capacitance as measured in a bridge circuit showed little change as the electrode was withdrawn from cochlear nucleus. Second, in one experiment the animal was sacrificed with an overdose of pentobarbital and sinusoidal signals (14 nA, peak current) from 500 to 5000 Hz were passed into the cochlear nucleus by a separate electrode placed a few hundred micra from the recording electrode. The recording electrode was withdrawn from the tissue and observed as for an ordinary run. Little change in phase was observed, a maximum of less than 10° at 6 kHz.

The latter control also eliminated the possibility that phase shifts observed in the FFR were caused by volume conduction of FFR currents through electrically reactive tissue. This confirmed our expectations, since the imaginary component of the impedance of neural tissue over the frequency range of the FFR is either negligible or very small in relation to the real component^{9,11}.

RESULTS

(1) Frequency response

The FFR can first be detected between 100 and 300 Hz, reaches its peak amplitude at about 1 kHz, and becomes quite small by 3 or 4 kHz. This upper limit is consistent with the findings of Worden and Marsh¹⁵, although the lower limit is considerably below the 800 Hz that they have reported. The detection of low frequency responses was facilitated in our work both by averaging and by subjecting the averaged data to Fourier spectral analysis.

An example of the power spectral density of the FFR at one point in the cochlear nucleus is shown in Fig. 1. The cochlear microphonic recorded from round window reproduces the stimulus tone with little distortion and good amplitude over the entire frequency range. In cochlear nucleus harmonics often appeared in response to low frequencies as can be seen in Fig. 1 in response to 500 Hz, while responses to 1–2 kHz were less contaminated by harmonics. Comparable harmonic distortion in the FFR to low frequency acoustic stimulation has been reported by Boudreau¹ in superior olive.

Fig. 1. Cochlear microphonic recorded from round window and frequency following responses recorded from cochlear nucleus to tonal signals from 500 to 5000 Hz. Averaged responses are on the left side of the figure and their root mean square (rms) power spectral densities on the right side. Time calibrations: 1 msec. Amplitude calibration: 100 μ V for microphonics and frequency following responses. Spectral density calibration mark represents 25 μ V for cochlear microphonics, and 5 μ V for cochlear nucleus. The position of the electrode is shown in the insert drawing. Animal FFR II. DC, dorsal cochlear nucleus; PV, posterior ventral cochlear nucleus; N VIII, eighth cranial nerve.

Fig. 2. Distribution of frequency following response every 250 μm along one electrode penetration in cochlear nucleus. Time marks are 5 msec. The location of the pass in cochlear nucleus is shown in Fig. 5, Pass A. Animal FFR VI, Pass A.

(2) Spatial distribution

The spatial distribution of the FFR along one pass through cochlear nucleus (Pass A, Fig. 5) is shown in the averaged responses in Fig. 2. Power and phase measurements of the FFR along this pass are plotted in Fig. 3. Although the specific shape of these distributions varied from experiment to experiment, we shall analyze this run in some detail as an example of the response behavior. At 100 Hz the amplitude distribution is rather broad, except for an abrupt null near the ventral border of the ventral cochlear nucleus. The phase plot shows a shift of about 0.5 cycle at this point but is otherwise constant. Such a distribution could be produced by a single localized generator or by a set of synchronous dipole generators located within a single plane and oriented with their axes perpendicular to that plane.

At 500 Hz the situation has changed. The power distribution shows 3 peaks (1.5, 2.5, and 4.5 mm below surface) while phase angle increases slowly with depth. Such a distribution could reflect activity in several stationary generators. For example, if two sinusoidal generators of the same frequency but of different phase were separated from each other in space, then at any given locus different percentages from each generator would sum to give a resultant wave that was sinusoidal, and with a phase intermediate between the two generators. The amplitude distribution for two generators would be bimodal, and the maximum phase shifts are possible. Hence at 500 Hz

Fig. 3. Distribution of root mean square power spectral density and phase angle of the frequency following response shown in Fig. 2. Animal FFR VI, Pass A.

a stationary distribution of generators that are out of phase could explain the data. Alternatively, a traveling wave like that generated along a nerve bundle might account for the data, as Tsuchitani and Boudreau¹³ have suggested for the FFR recorded in the trapezoid body. However, in the case of a traveling wave one should expect phase angle to change linearly with distance, and the slope of this line to be directly proportional to the frequency. We did not observe these relations (Fig. 3), but our failure to do so does not exclude the traveling wave hypothesis. A linear phase profile would be observed in a wave traveling down an orderly array of parallel fiber bundles. In cochlear nucleus, however, VIIIth nerve fibers sensitive to different portions of the acoustic frequency range are distributed in a complex spiral fashion to different parts of the nucleus¹² complicating the fields recorded with our penetrating electrode.

By 1000 Hz the phase angle shift had reversed and now decreased linearly as the electrode penetrates the nucleus. Such a decrease in phase was the exception as the other 3 animals studied showed an increase in phase angle with electrode penetration. The response amplitude is small in the dorsal cochlear nucleus, becoming large and level in the ventral cochlear nucleus.

By 2000 Hz the response is virtually zero in the dorsal cochlear nucleus and reaches a maximum at the very base of the cochlear nucleus and in the VIIIth nerve itself. Phase angle continues to decrease gradually with depth. The apparent phase angle increase in the first 3 points located in dorsal cochlear nucleus is not significant since phase angle measurements are subject to large errors when the amplitude of the response is small.

The amplitude of the FFR but not its phase was affected by the position of the tract within the nucleus. In Fig. 4 the spectral power and phase distributions for 3 other passes from the same experiment shown in Figs. 2 and 3 are plotted along with those in Fig. 3. Histology for these passes is shown in Fig. 5.

Each stimulus frequency evoked markedly different distributions for the FFR. Yet, at any one frequency, the spatial distributions of power and phase were somewhat similar in all 4 passes. Hence it appears that for a given frequency the FFR changes regularly in the dorso-ventral direction in cochlear nucleus relatively independent of the position of the electrode in the horizontal (antero-posterior and latero-medial) planes of the nucleus.

(3) Stimulus intensity

FFR power and phase distributions in response to 4 stimulus intensities are shown in Fig. 6. Stronger stimulation increases the amplitude of the response and

Fig. 4. Power spectral density and phase angle of frequency following response from 4 different passes through cochlear nucleus in one animal (FFR VI), as is shown in Fig. 5.

Fig. 5. Tracings of the 4 passes through cochlear nucleus (A, B, C, D) in Animal FFR VI from which data are represented in Figs. 1, 3, and 4. DC, dorsal cochlear nucleus; AV, anterior ventral cochlear nucleus; PV, posterior ventral cochlear nucleus; N VIII, eighth cranial nerve; RB, restiform body.

shifts its peak somewhat in the nucleus, but phase relations remain relatively unchanged provided that the amplitude of the response is sufficiently large to minimize the effects of noise and of potentials projected from distant structures.

(4) *Relation to click evoked responses*

The amplitude distribution of the FFR within the cochlear nucleus paralleled the amplitude distribution of the click evoked response. However, it was difficult to define any consistent latency shift in the components of the click evoked response that paralleled the phase shifts described for the FFR.

DISCUSSION

The present study demonstrates that the FFR is distributed in a complex manner through cochlear nucleus. In general the power spectrum of the FFR shows both broad and narrow peaks and troughs, with most of the power concentrated in the ventral cochlear nucleus and VIIIth nerve. The phase angle of the response changes

Fig. 6. Power spectral density and phase angle of frequency following response as a function of tone intensity. —20 dB is reference voltage level to earphone at which tone evoked 50–100 μ V (peak to peak measurement) of cochlear microphonic recorded at round window. Animal FFR V.

gradually in a dorso-ventral direction and may suffer 0.5 cycle phase shifts corresponding to a trough or null in the power spectrum. Stimulus intensity affects amplitude profile more than phase distributions within the nucleus. Other investigators of the FFR have noted variability of this response from animal to animal^{13,14}, and our experience is no exception.

Sando¹² has shown that the VIIIth nerve enters the ventral aspect of the cochlear nucleus and rises dorsally, twisting as it goes. As it rises it gives off fibers which bifurcate and spread horizontally through the cochlear nucleus in the same horizontal plane in which the fibers leave the nerve. These fibers are frequency specific and tonotopically arranged, the more ventral fibers arising from the apical or low frequency end of the cochlea, the more dorsal fibers from the basal or high frequency end. The cochlear nucleus is thus not likely to show much response variation within any particular horizontal plane, whereas measurements taken from all ventrodorsal passes should have similar characteristics at a given frequency, and these characteristics should be sharply frequency dependent. This is precisely what we have observed. However, we have not been able to find an electrophysiological correlate in the FFR to the specific tonotopic distribution of afferent VIIIth nerve fibers in cochlear nucleus. For example, the locus at which the FFR is of maximal amplitude did not change in an orderly fashion as a function of stimulus frequency. There are several possible ex-

planations for this. First, if the FFR is generated as an envelope of nerve action potentials, then the nature and location of the current sources are uncertain and variable, especially since the volume conducted nerve potential in cochlear nucleus changes from the field of a traveling impulse to the field associated with a terminal bouton⁶. If, on the other hand, the FFR potentials are synaptic in origin, then the distribution and orientation of the dendrites of the postsynaptic cells would strongly affect the apparent source location seen by the probing electrode. In cochlear nucleus dendritic patterns are random except for the alignment of the pyramidal cell dendrites in dorsal cochlear nucleus¹⁰, the site in which the FFR was smallest.

One additional complication in interpreting the spatial distribution of the FFR in cochlear nucleus is that time is required for acoustic signals to traverse the basilar membrane thus causing a temporal dispersion of activity in VIIIth nerve fibers. For example, low frequency acoustic signals will activate fibers originating in the basilar and middle turns of the cochlea some 2–5 msec before activating fibers from the apex⁵. The volley of activity recorded from the VIIIth nerve is not composed of synchronous nerve firings but rather of firings that have the same periodicity but a variable phase relationship to one another. If the FFR is in fact an envelope of action potentials, the dispersion of VIIIth nerve firings over time may account for harmonic distortion of the FFR found in response to low frequency acoustic signals. Certainly a dispersion of VIIIth nerve input both in time and in space to cochlear nucleus complicates any simple explanation of the FFR recorded from this structure.

Two approaches for further defining the the generating mechanisms of the FFR might include:

(1) Degeneration studies, in which the brain stem acoustic stria, principally the trapezoid body, are destroyed leading to retrograde degeneration of cells in cochlear nucleus. If the FFR were generated postsynaptically, the loss of cells would be accompanied by an attenuation of the FFR. However, if the FFR were an envelope of VIIIth nerve action potentials, then this procedure would be without effect.

(2) Antidromic stimulation of the trapezoid body with short current pulses during low frequency driving of the FFR. If the FFR were generated postsynaptically, it would be affected by the antidromic invasion of the soma-dendritic regions of cochlear nucleus cells. If the response were an action potential envelope then there should be little interaction between the FFR and antidromic activity and the two signals should appear additive.

SUMMARY

We have investigated the frequency following response (FFR) to pure tones in the cochlear nucleus of anesthetized cats with regard to the spatial distribution of amplitude and phase.

Amplitude distributions were variable from animal to animal and showed a range of responses including single and multiple peaks and nulls, broad plateaus, and gradually tapering slopes. In general, amplitudes were larger in the ventral cochlear nucleus and VIIIth nerve than in dorsal cochlear nucleus.

Phase distributions displayed gradual phase shifts across the nucleus as well as abrupt phase changes in the order of 0.5 cycle.

Stimulus frequency had a greater effect upon the dorso-ventral profile of both phase and amplitude of the FFR than did the horizontal position of the electrode track within the cochlear nucleus.

Changing stimulus intensity altered the amplitude profile of the FFR at a given frequency but had relatively little effect upon the phase distribution.

Our data are consistent with the following hypotheses: (1) the FFR is generated as an envelope of the VIIIth nerve action potentials as the nerve arborizes and terminates in the cochlear nucleus. Phase shifts may be caused by traveling action potentials along the nerve and/or by volume conduction from different fiber bundles to regions lying between these bundles; (2) the FFR is generated postsynaptically in cochlear nucleus cells innervated by the VIIIth nerve. Once again, phase shifts may reflect the sequential innervation of adjacent dendrites, a 'traveling' postsynaptic wave, or the response of a few generators at different loci driven at different phases, in which case spatial phase shifts are caused by the summing of responses as currents are volume conducted to the recording electrode.

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