clusions (Fig. 1, b to d). These cells contained oil red O–stained lipid droplets (Fig. 1a) similar to the oil red O–stained lipid droplets that accumulate in smooth muscle cells within atherosclerotic lesions (*5, 6*). Examination of the cultured smooth muscle cells, prepared for electron microscopic analysis as described (*11*), revealed that non-membrane-bound lipid droplets had accumulated. Addition of increasing numbers of platelets (7.5×10^8, 15×10^8, and 30×10^8 per culture) resulted in increasing amounts of cholesteryl ester within cells. The response of nonconfluent cultures of smooth muscle cells was similar to that of confluent cultures. No lipid inclusions accumulated in smooth muscle cells from cultures incubated with unactivated platelets (that is, in the absence of thrombin) (Fig. 1, e and f) or from cultures incubated with thrombin in the absence of platelets (data not shown).

The possibility that thrombin-activated platelets release a factor that induces cholesteryl ester lipid droplet accumulation in smooth muscle cells was tested. Washed platelets (30×10^8 in 0.5 ml of DPBS plus glucose) were added to 3.5 ml of MEM containing 0.35 percent bovine serum albumin in single-well slide chambers. These platelet cultures were incubated with or without thrombin for 24 hours in the absence of smooth muscle cells. Supernatants from these cultures were collected and then centrifuged at 1000*g* for 3 minutes and filtered (0.4 μm pore) to remove all platelets. These platelet-free supernatants when added to and incubated for 24 hours with smooth muscle cells caused cholesteryl ester lipid droplet accumulation (Fig. 1, g and h). Supernatants centrifuged at 10,000*g* for 10 minutes also induced cholesteryl ester lipid droplet accumulation in smooth muscle cells. Supernatants from platelets incubated without thrombin and then added to and incubated with smooth muscle cell cultures did not promote cholesteryl ester lipid droplet accumulation (Fig. 1, i and j).

Supernatants from platelets incubated with thrombin for increasing lengths of time (0.5, 1, 3, or 24 hours) before being added to and incubated for 24 hours with smooth muscle cell cultures promoted progressively increasing amounts of cholesteryl ester lipid droplet accumulation. However, supernatants from platelets incubated with thrombin for 48 hours before addition to smooth muscle cell cultures did not promote greater lipid droplet accumulation than did supernatants form platelets incubated with thrombin for 24 hours.

Addition of the cholesterol synthesis inhibitor mevinolin (10 μ*M*) (*12*) to smooth muscle cell cultures incubated with thrombin-activated platelets did not diminish cholesteryl ester lipid droplet accumulation. Hence, cholesteryl ester lipid droplet accumulation does not appear to depend on de novo cholesterol synthesis. Also, since platelet-free supernatants could induce cholesteryl ester lipid droplet accumulation, lipid droplet accumulation did not result simply from endocytosis of activated platelets by the smooth muscle cells. It is possible that thrombin-activated platelets release cholesterol in some form that can be taken up by smooth muscle cells and stored as cholesteryl ester lipid droplets. This possibility is supported by previous studies that showed release of cholesterol-containing lipoprotein complexes from thrombin-activated platelets (*13, 14*).

In addition to accumulating in smooth muscle cells, cholesterol accumulates within macrophages in atherosclerotic lesions. Furthermore, thrombin-activated platelets cause cholesteryl ester accumulation within cultured macrophages (*15*). Thus, platelet-mediated cholesteryl ester lipid droplet accumulation in macrophages and vascular smooth muscle cells may be related to cellular cholesterol accumulation in these cells within atherosclerotic lesions.

Induction of vessel-associated thrombi in experimental animals is associated with the development of lipid-containing atherosclerotic lesions (*16–20*). This occurs even when serum cholesterol levels are not high. Lesion development is inhibited in these animals when they are made thrombocytopenic (*20*).

The possible role of platelets in promoting smooth muscle cell proliferation within atherosclerotic lesions was recognized earlier (*21*). Since it has now been shown that platelets can mediate cholesteryl ester lipid droplet accumulation within cultured vascular smooth muscle cells, the possible role of platelets in cholesterol accumulation within atherosclerotic lesions must be considered in evaluating the pathogenesis of atherosclerosis.

HOWARD S. KRUTH
Laboratory of Experimental Atherosclerosis, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

References and Notes

1. S. Weinhouse and E. F. Hirsch, *Arch. Pathol.* **29**, 31 (1940).
2. M. D. Haust, R. H. More, H. Z. Movat, *Am. J. Pathol.* **37**, 377 (1960).
3. J. C. Geer, H. C. McGill, Jr., J. P. Strong, *ibid.* **38**, 263 (1961).
4. G. L. Duff, G. C. McMillan, A. C. Ritchie, *ibid.* **33**, 845 (1957).
5. H. S. Kruth, *ibid.* **114**, 201 (1984).
6. ———, *Lab. Invest.* **50**, 87 (1983).
7. R. Ross and B. Kariya, in *Handbook of Physiology*, D. F. Bohr, Ed. (American Physiological Society, Bethesda, Md., 1980), vol. 2, section 2, p. 69.
8. H. S. Kruth and M. Vaughan, *J. Lipid Res.* **21**, 123 (1980).
9. H. S. Kruth, J. Avigan, W. Gamble, M. Vaughan, *J. Cell Biol.* **83**, 588 (1979).
10. H. S. Kruth, *Atherosclerosis* **51**, 281 (1984).
11. ———, J. Blanchette-Mackie, J. Avigan, W. Gamble, M. Vaughan, *J. Lipid Res.* **23**, 1128 (1982).
12. A. W. Alberts *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3957 (1980).
13. M. G. Davey and E. F. Lüscher, *Biochim. Biophys. Acta* **165**, 490 (1968).
14. H. Sandberg, L. O. Andersson, S. Höglund, *Biochem. J.* **203**, 303 (1982).
15. H. S. Kruth, unpublished observation.
16. S. Björkerud and G. Bondjers, *Atherosclerosis* **18**, 235 (1973).
17. S. Moore, *Lab. Invest.* **29**, 478 (1973).
18. R. J. Friedman, S. Moore, S. P. Singal, *ibid.* **30**, 404 (1975).
19. A. Sumiyoshi, R. H. Moore, B. I. Weigensberg, *Atherosclerosis* **18**, 43 (1973).
20. S. Moore and I. O. Ihnatowycz, *Adv. Exp. Med. Biol.* **102**, 145 (1977).
21. R. Ross, J. Glomset, B. Kariya, L. Harker, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1207 (1974).
22. I thank G. Johnson, F. Carter, and R. Seabron for technical assistance; C. Kosh for help in preparation of the manuscript; the Upjohn Company for providing filipin; A. W. Alberts (Merck and Company) for providing mevinolin and W. Lovenberg and T. Nabika for providing the smooth muscle cell line.

5 October 1984; accepted 9 November 1984

Auditory Midbrain Responses Parallel Spectral Integration Phenomena

Abstract. *Resolving the frequency components of complex sound spectra including speech is an inherent, important accomplishment of the auditory nervous systems of vertebrates. The critical perceptual unit in the frequency domain, the critical bandwidth, has a simple functional equivalent within the principal midbrain auditory nucleus—the central nucleus of the inferior colliculus.*

The study of the resolution and interaction of components of complex sound spectra has been essential for understanding the basis of the perception of frequency spectra, for example, in speech, in music, and in animal vocalizations (*1*). Frequency resolution in the auditory system has been defined in various psychophysical tests. Common measures obtained are frequency bandwidths crucial for the perception of the quality of the sound stimulus, called "critical bands." Critical bands have been described as "filters" in the auditory sys-

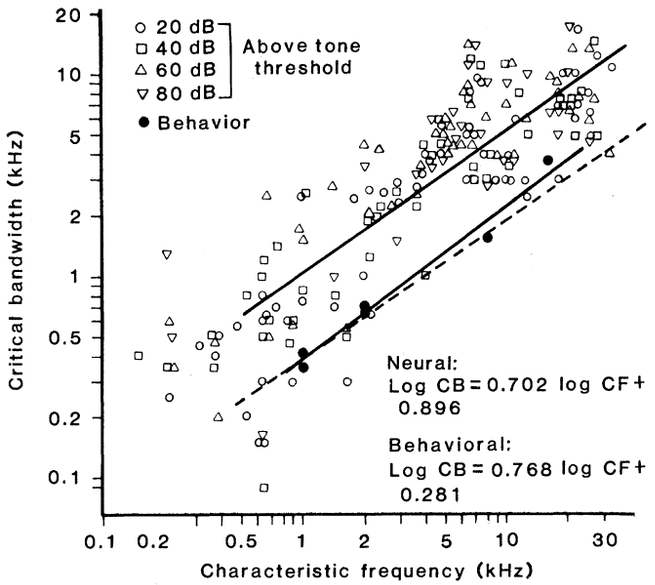

Fig. 1. Frequency dependence of critical bandwidths of single ICC units (open symbols) and from behavioral tests (closed symbols) (*9*). Regression lines of neural and behavioral data are shown. The dashed line is the neural regression line adjusted to compensate for overmasking (*14*). Results from different cats showed no obvious clustering in this distribution. All units were within the ICC.

tem that divide a sound spectrum into units of perception (*2*). The perceived sound quality changes when information distributed within critical band filters is altered. For example, (i) loudness summation of sound components occurs only within a critical band (*3*); (ii) noise effective in masking a tone is concentrated within a critical band of frequency around the tone frequency (*2*); (iii) phase relationships of an amplitude-modulated sound are detected only when spectral components are within a critical band (*4*); (iv) the transition from consonance to dissonance in a tone complex arises with frequency separations of components on the order of a critical bandwidth (*5*); (v) categorical changes in identification of speech sounds and animal vocalizations occur when component frequency ratios vary by a distance roughly corresponding to a critical bandwidth (*6*); and (vi) sound lateralization images are fused only when input component frequencies fall within a critical bandwidth (*7*).

Surprisingly, neither the location nor the nature of neurophysiological mechanisms accounting for critical bandwidth phenomena are well understood. Several simple indices of neural response would be expected to be evident at sites of origin of critical bandwidths. First are the predicted bandwidths themselves and a long-defined relationship between bandwidths and their center frequencies (*1–3, 8, 9*). Second, filter bandwidths are independent of stimulus intensity up to high sound pressure levels (*2, 7*). To determine the sites of origin and basic nature of the neural representation of critical band information, these two properties have been studied initially in the principal auditory nucleus in the midbrain, the central nucleus of the inferior colliculus (ICC). Input from the auditory nerve cannot account for the independence of critical band phenomena from loudness (*10, 11*). Information is distributed from the auditory nerve to many nuclear subdivisions and nuclei. All converge in projection to the ICC (*12*). Thus, if it is accepted that critical bandwidth phenomena are not completely accounted for in the responses of auditory nerve fibers, then either frequency resolution measured in hearing tests is a product of many different, parallel neural processing pathways (which seems unlikely), or it arises at or above this first convergent auditory center.

This study includes data from 73 quantitatively studied single neurons located in the ICC of seven adult cats (*13*). To facilitate comparison with psychophysical measurements in cats (*9*), we used the same stimuli and a procedure similar to that used in behavioral measurements of critical bands. After a neuron's characteristic frequency (CF) was defined, tonal pulses were delivered in the presence of continuous broadband noise. Noise intensity was increased until the neural responses to tones were no longer detectable (*10, 13*). This masked threshold of the neural response to tones by broadband noise was defined at tone levels 20, 40, 60, and 80 dB (relative to 20 μPa) above neural response thresholds at CF.

At the masked threshold for a tone of given intensity, the noise was then bandpass filtered (48 dB per octave slopes) and the upper and lower cutoff frequencies changed to create a progressively narrower noise band around the tone. The objective was to define the critical bandwidth at which a response to the tone again emerged. To evaluate the intensity independence of critical band measures, they were derived, again, at 20, 40, 60, and 80 dB above their CF thresholds.

The frequency dependence of single-unit critical bandwidths at all intensities is shown with behaviorally obtained values (*9*) in Fig. 1. In this log-log plot, bandwidths of neural and psychophysically defined critical band filters increase systematically as a function of the center frequency of the filters. The regression lines of both sets of data indicate a statistically significant correlation ($P < 0.001$), and as slope values are similar, we conclude that the frequency dependence of critical bandwidths in the auditory midbrain (ICC) is essentially the same as that measured in the behaving animal. The wider-than-behavioral estimation of critical bandwidths for many studied neurons shows that behavioral masking requires overmasking for most individual neurons, as it must result in the complete masking of the population least sensitive to it. Consistent with this view, behavioral bandwidths coincided with the narrowest defined neural values. When the average neural data were adjusted for overmasking (*14*), behavioral and neural regression lines closely overlie one another.

For neurons with CF's below and above 3 kHz, neural critical bandwidths are independent of sound intensity (Fig. 2). No significant differences among the values as a function of intensity could be detected (Mann-Whitney *U* test), and a regression analysis revealed no signifi-

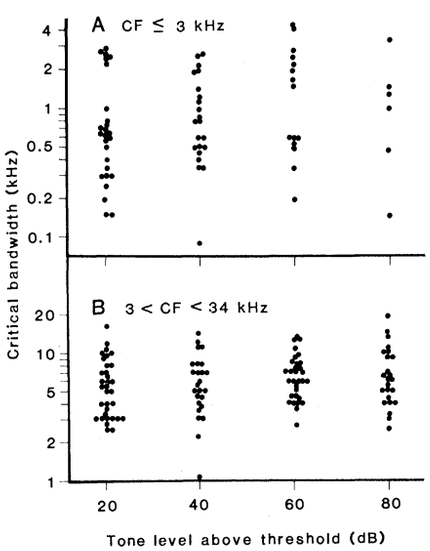

Fig. 2. Critical bandwidths of single units in the ICC plotted as a function of the tone intensity above response thresholds for neurons with characteristic frequencies below (A) or above (B) 3 kHz.

cant correlation between bandwidth and intensity. Thus, we conclude that the independence of stimulus intensity and behaviorally measured critical bandwidths up to about 80 dB (*7*) is paralleled by independence of stimulus intensity and the averaged neural filter bandwidths recorded in the ICC.

Our data indicate that two key indices of the frequency resolving capability of the auditory system, namely critical bandwidth as a function of frequency and intensity independence of critical band measures, also describe the frequency-selective responses of ICC neurons (*15*). These results have several important implications for auditory signal processing in the brain and for sound perception in general. First, a frequency dependence of auditory filter bands with a general form similar to that shown in Fig. 1 is recorded for auditory nerve fibers (*11, 16*). However, numerical values of critical bandwidths of cochlear nerve fibers defined with these simultaneous masking techniques are only about 1/3 the psychophysically determined critical bandwidth (*9, 11*), and response filtering in the nerve is not independent of intensity (*10, 16*). The interaction phenomena underlying these properties probably arise at the level of the ICC. Alternatively, the lateral inhibition required to establish these phenomena is effected on the manifold parallel pathways to the ICC. That would seem unlikely. Neuroscientists have puzzled over the significance of the complex convergence of inputs of many auditory nuclei (more than ten) to the ICC (*12*). Our data indicate that critical bandwidth phenomena are established, within this nucleus, for all of these inputs. That explains how critical band phenomena apply in a similar way to sound processing in very different perceptual domains, for example, for the representation of pitch, loudness, the recognition of complex spectra, and in sound localization.

For any given frequency, a range of critical bandwidths was recorded for different neurons. The significance of this finding is indicated by recent studies revealing an orderly representation of best modulation frequencies within the "isofrequency laminae" of the ICC (*17*). Obvious signal processing advantages are inherent in varying the integration window of different neurons within this system.

GÜNTER EHRET*
MICHAEL M. MERZENICH
Coleman Laboratory,
Department of Otolaryngology,
University of California,
San Francisco 94143

References and Notes

1. R. Plomp, *Aspects of Tone Sensation* (Academic Press, London, 1976); E. Zwicker and R. Feldtkeller, *Das Ohr als Nachrichtenempfanger* (Hirzel, Stuttgart, 1967).
2. B. Scharf, in *Foundations of Auditory Theory*, J. V. Tobias, Ed. (Academic Press, New York, 1970), pp. 159–202; H. Fletcher, *Rev. Mod. Phys.* **12**, 47 (1940).
3. E. Zwicker, G. Flottorp, S. S. Stevens, *J. Acoust. Soc. Am.* **29**, 548 (1957).
4. E. Zwicker, *Acustica Akust. Beih.* **3**, 125 (1952).
5. R. Plomp and W. J. M. Levelt, *J. Acoust. Soc. Am.* **38**, 548 (1965).
6. G. Ehret and B. Haack, *J. Comp. Physiol.* **148**, 245 (1982); J. C. Flanagan, *Speech Analysis* (Springer-Verlag, Berlin, 1972).
7. B. Scharf and C. H. Meiselman, in *Psychophysics and Physiology of Hearing*, E. F. Evans and J. P. Wilson, Eds. (Academic Press, London, 1977), pp. 221–232; B. Scharf, M. Florentine, C. H. Meiselman, *Sens. Processes* **1**, 109 (1976).
8. D. D. Greenwood, *J. Acoust. Soc. Am.* **33**, 484 (1961).
9. J. O. Pickles, *Acta Otolaryngol.* **80**, 245 (1975); *J. Acoust. Soc. Am.* **66**, 1725 (1979).
10. G. Ehret and A. J. M. Moffat, *Hearing Res.*, in press.
11. J. O. Pickles and S. D. Comis, *J. Acoust. Soc. Am.* **60**, 1151 (1976).
12. G. L. Roth, L. M. Aitkin, R. A. Andersen, M. M. Merzenich, *J. Comp. Neurol.* **182**, 661 (1978); B. Beyerl, *Brain Res.* **145**, 209 (1978); H. Schweizer, *J. Comp. Neurol.* **201**, 25 (1981); F. H. Willard and D. K. Ryugo, in *The Auditory Psychobiology of the Mouse*, J. F. Willot, Ed. (Thomas, Springfield, Ill., 1983), pp. 201–304; M. M. Merzenich, W. M. Jenkins, J. C. Middlebrooks, in *Dynamic Aspects of Neocortical Function*, G. Edelman *et al.*, Eds. (Wiley, New York, 1984), pp. 397–424.
13. Animals were anesthetized with sodium pentobarbital and chlorprotixen (Taractan). Temperature was maintained at 37°C. Studies were conducted in a sound-isolated chamber. Sound stimuli were delivered through sealed ear bars according to a calibrated sound system with a flat (±6 dB) frequency response from 100 Hz to 26 kHz. Tone bursts were 200 msec long, with 10-msec rise and fall times, repeated with 500-msec duty cycles. Masking noise stimuli were continuous. The dorsal surface of the inferior colliculus was exposed, and paralene-insulated tungsten electrodes were advanced into the central nucleus to record from single isolated units. Basic binaural and monaural response properties of all neurons were defined. After deriving a single unit's tuning curve, we measured the masked threshold for broadband noise. Noise was then bandpassed and bands narrowed progressively to define critical bandwidths. In these parametric stepwise stimulus series, each stimulus was repeated 20 times and spike counts and peristimulus time histograms were generated. All described measures were derived from these data. Comparable critical bandwidths were recorded for nearly all of the distinctive functional classes of neurons (*12*) within the ICC.
14. The average difference between tone and noise levels at which the response of single neurons within the ICC were masked was 4.5 dB greater than was required for behavioral masking. That is, in reference to behavioral measures, the average ICC neuron was overmasked by 4.5 dB. This 4.5-dB difference is equal to a factor of 2.8 for bandwidth (*2, 11*) (Fig. 1).
15. This is true for critical bandwidths determined in narrowband noise-masking tests. No other measures of critical bands have yet been described in the responses of ICC neurons.
16. A. R. Moller, *Acta Physiol. Scand.* **78**, 289 (1970); E. F. Evans and J. P. Wilson, in *Basic Mechanisms in Hearing*, A. R. Moller, Ed. (Academic Press, New York, 1973), pp. 519–551.
17. C. Schreiner and G. Langner, *Soc. Neurosci. Abstr.* **10**, 395 (1984).
18. We thank W. Jenkins, C. Schreiner, and R. Snyder for technical assistance. Supported by grants NS-10414 and Eh 53/7-1 from the Deutsche Forschungsgemeinschaft, and by HRI and the Coleman Fund.
* Present address: Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany.

30 May 1984; accepted 25 October 1984

Increased Plasma Interleukin-1 Activity in Women After Ovulation

Abstract. *The polypeptide interleukin-1 mediates many host responses to infection and inflammation. A method was developed for studying interleukin-1 levels in human plasma from febrile patients. Interleukin-1 activity was also consistently found in plasma samples from women in the luteal phase of their menstrual cycle. This activity was neutralized by a specific antiserum to human interleukin-1 and was low in plasma from healthy men and preovulatory women. Thus interleukin-1 appears to have a role in normal physiological conditions as well as in disease states.*

In response to infection, injury, and certain immunological reactions, specialized cells (including monocytes, keratinocytes, and synovial cells) release interleukin-1 (IL-1). This protein, or family of closely related proteins, mediates the acute-phase responses to infection. These responses include fever, redistribution of amino acids and trace metals, and accelerated hepatic synthesis of certain plasma proteins. In addition, IL-1 has potent immunological properties, such as promoting lymphocyte proliferation and stimulating production of lymphokines (*1*).

The biological role of IL-1 in host defense has been inferred from studies of its influence on cell cultures in vitro and its systemic effects after it is injected into laboratory animals. Although substances exhibiting IL-1 activity have been isolated from human peritoneal and synovial fluid (*2*), correlation of IL-1 levels in the circulation with various physiological or pathological conditions is difficult with existing assay methods (*3*) and is complicated by interfering plasma factors (*4*).

We report a simple and direct method for detecting IL-1 activity—after removal of interfering factors—by rapid (<30 minutes) gel filtration (*5*). IL-1 activity was determined with the well-established lymphocyte-activating factor (LAF) assay, in which augmentation of mitogen-induced proliferation of murine thymocytes is measured. Using this method, we have consistently observed increased plasma IL-1 activity after ovulation as well as during infection.

Blood from healthy subjects and from