yond S_6 (*23*, *24*) contribute to the inner mouth of the pore, while residues in the 5′ region of S_6 contribute to the outer mouth (*25*). In IRKs, the pore seems to have a major contribution from the carboxyl terminus, with H_5 as well as M_1 and M_2 having smaller roles in K^+ conduction and inward rectification. Although the pore of IRKs may also be made up of several segments of the protein, our results call for a reassessment of the current folding model of IRK channels to account for their unique permeation and blocking properties.

REFERENCES AND NOTES

1. K. Ho *et al.*, *Nature* **362**, 31 (1993).
2. Y. Kubo, T. Baldwin, Y. N. Jan, L. Y. Jan, *ibid.*, p. 127.
3. Y. Kubo, E. Reuveny, P. A. Slesinger, Y. N. Jan, L. Y. Jan, *ibid.* **364**, 802 (1993).
4. N. Dascal *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10235 (1993).
5. D. M. Papazian, T. L. Schwarz, B. L. Tempel, Y. N. Jan, L. Y. Jan, *Science* **237**, 749 (1987); A. Baumann *et al.*, *EMBO J.* **6**, 3419 (1987); A. Kamb, L. E. Iverson, M. A. Tanouye, *Cell* **50**, 405 (1987).
6. B. Tempel, Y. N. Jan, L. Y. Jan, *Nature* **332**, 837 (1988); H. R. Guy and F. Conti, *Trends Neurosci.* **13**, 201 (1990); S. R. Durell and H. R. Guy, *Biophys. J.* **62**, 238 (1992).
7. H. A. Hartmann *et al.*, *Science* **251**, 942 (1991); R. MacKinnon and G. Yellen, *ibid.* **250**, 276 (1990); G. Yellen, M. E. Jurman, T. Abramson, R. MacKinnon, *ibid.* **251**, 939 (1991); A. J. Yool and T. L. Schwarz, *Nature* **349**, 700 (1991).
8. M. Noda, H. Suzuki, S. Numa, W. Stuhmer, *FEBS Lett.* **259**, 213 (1989); S. Heinemann, H. Terlau, W. Stuhmer, K. Imoto, S. Numa, *Nature* **356**, 441 (1992); J. Satin *et al.*, *Science* **256**, 1202 (1992).
9. S. Tang *et al.*, *J. Biol. Chem.* **268**, 13026 (1993); P. T. Ellinor *et al.*, *Nature* **363**, 455 (1993).
10. E. H. Goulding, G. R. Tibbs, D. Liu, S. A. Siegelbaum, *Nature* **364**, 61 (1993).
11. C. G. Nichols, *Trends Pharmacol. Sci.* **14**, 320 (1993).
12. ROMK1 complementary DNA (cDNA) was obtained by reverse transcriptase–polymerase chain reaction (RT-PCR) by the use of rat kidney total RNA as template. The ROMK1 cDNA with minimal 5′ and 3′ untranslated sequences was subcloned into the PCRII vector (Invitrogen, San Diego, CA) and sequenced completely in both directions. The predicted amino acid sequence is identical to the published ROMK1 sequence (*2*). A portion of the rat DRK1 cDNA 3′ untranslated region including the polyadenylated (poly A^+) tail was ligated to the 3′ end of the ROMK1 cDNA (ROMK1-A^+-PCRII) to boost expression of the mRNA in *Xenopus* oocytes. Chimeric cDNA constructs between ROMK1 and IRK1 were prepared by overlap extension at the junctions of the relevant domains by the use of sequential PCR [R. M. Horton, H. D. Hunt, S. N. Ho, J. K. Pullen, L. R. Pease, *Gene* **77**, 61 (1989)]. The resulting PCR products were subcloned into PCRII and sequenced to verify the correct chimeric construction. The chimeric constructs A, B, C, and N were subcloned as Apa I–Eco RI fragments into PCRII containing the rat DRK1 3′ untranslated–poly A^+ tail region. The reverse chimeric constructs A and B, with IRK1 regions transplanted into ROMK1, were subcloned as Apa I–Sal I fragments into ROMK1-A^+-PCRII from which the corresponding wild-type ROMK1 fragment had been removed. Complementary RNAs for injection into *Xenopus* oocytes were prepared by the use of the mMESSAGE mMACHINE kit (Ambion) with T7 RNA polymerase after linearization of the plasmids with Bam H1 (for ROMK1) or Not I (for IRK1). Stage V to VI *Xenopus* oocytes were injected with 46 nl of cRNA (2 to 100 ng/μl) dissolved in 0.1 M KCl [M. Taglialatela *et al.*, *Pfluegers Arch.* **423**, 104 (1993)]. Single-channel currents were recorded at room temperature (22° to 24°C) from membrane patches with fire-polished and Sylgard-coated (Dow-Corning, Midland, MI) micropipettes of 2- to 5-megohm resistance. The pipette solution was K^+-Ringer [100 mM KCl, 2 mM $MgCl_2$, 10 mM Hepes (pH 7.3)], while the bath solution was iso-K^+ [100 mM KCl, 10 mM EGTA, 10 mM Hepes (pH 7.3)]. $MgCl_2$ was added to the bath solution to give calculated concentrations of free Mg^{2+} ions [A. Fabiato and F. Fabiato, *J. Physiol.* (*Paris*) **74**, 463 (1979)]. Data were low-pass filtered at 1 to 2 kHz (−3 dB, 4-pole Bessel filter) before digitization at 5 to 10 kHz. The pClamp software (Axon Instruments) was used for the generation of the voltage-pulse protocols and for data acquisition. Ramp-stimulated currents (0.5 to 2 mV/ms) were analyzed as described [G. E. Kirsch *et al.*, *Biophys. J.* **62**, 136 (1992)]. Macroscopic currents were recorded with a conventional two-microelectrode voltage-clamp technique [M. Taglialatela *et al.*, above]. For macroscopic current recordings, bath solutions were as follows: low K^+-MES [2.5 mM KOH, 120 mM *N*-methyl-D-glucamine, 122.5 mM MES, 2 mM $Mg(OH)_2$, and 10 mM Hepes (pH 7.3)]; intermediate K^+-MES [22.5 mM KOH, 100 mM *N*-methyl-D-glucamine, 122.5 mM MES, 2 mM $Mg(OH)_2$, and 10 mM Hepes (pH 7.3)]; or high K^+-MES [100 mM KOH, 22.5 mM *N*-methyl-D-glucamine, 122.5 mM MES, 2 mM $Mg(OH)_2$, and 10 mM Hepes (pH 7.4)]. Statistical analysis was performed by means of the two-tailed Student's *t* test for samples of unequal variances.
13. H. Matsuda, A. Saigusa, I. Irisawa, *Nature* **325**, 156 (1987).
14. C. A. Vandenberg, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2560 (1987).
15. K. Ishihara, T. Mitsuye, A. Noma, A. M. Takano, *J. Physiol.* (*London*) **419**, 297 (1989).
16. H. Matsuda, *ibid.* **397**, 237 (1988).
17. M. R. Silver and T. E. DeCoursey, *J. Gen. Physiol.* **96**, 109 (1990).
18. C. G. Nichols, K. Ho, S. Hebert, *Proc. Physiol. Soc.* **1993**, 46 (1993).
19. M. J. Root and R. MacKinnon, *Neuron* **11**, 459 (1993).
20. E. Y. Isacoff, Y. N. Jan, L. Y. Jan, *Nature* **353**, 86 (1991); P. A. Slesinger, Y. N. Jan, L. Y. Jan, *Neuron* **11**, 739 (1993).
21. G. E. Kirsch, C.-C. Shieh, J. A. Drewe, D. F. Verner, A. M. Brown, *Neuron* **11**, 503 (1993).
22. K. L. Choi, C. Mossman, J. Aube', G. Yellen, *ibid.* **10**, 533 (1993).
23. G. A. Lopez, Y. N. Jan, L. Y. Jan, *Nature* **367**, 179 (1994).
24. M. Taglialatela *et al.*, *J. Biol. Chem.*, in press.
25. T. Hoshi, W. N. Zagotta, R. W. Aldrich, *Neuron* **7**, 547 (1991).

26. We thank L. Jan for providing the IRK1 clone; W.-Q. Dong for injecting and handling oocytes; T. Afinni, C. Zhu, S. Dou, and S. Pan for expert molecular biology support; and G. E. Kirsch and A. E. Lacerda for helpful comments. Supported by NIH grants NS23877, HL36930, and HL37044 (A.M.B.).

31 January 1994; accepted 21 March 1994

Neural Tuning for Sound Duration: Role of Inhibitory Mechanisms in the Inferior Colliculus

J. H. Casseday,* D. Ehrlich, E. Covey

Duration is a biologically important feature of sound. Some neurons in the inferior colliculus of the big brown bat, *Eptesicus fuscus*, are tuned to sound duration, but it is unclear at what level the tuning originates or what neural mechanisms are responsible for it. The application of antagonists of the inhibitory neurotransmitters γ-aminobutyric acid or glycine to neurons in the inferior colliculus eliminated duration tuning. Whole-cell patch-clamp recordings of synaptic currents suggested that inhibition produces a temporal frame within which excitation can occur. A model is proposed in which duration tuning arises when an early, sustained inhibitory input interacts with a delayed, transient excitatory input.

The duration of a sound is a signature of biological importance, particularly in speech or echolocation signals. The perception of speech patterns, including the silent gaps between sounds, operates within time constraints in such a way that the same sounds are perceived differently, depending on their duration (*1*). The signals emitted by echolocating bats vary in duration according to the phase of hunting in which the bat is engaged. The big brown bat systematically varies the duration of its echolocation call during pursuit of flying prey; the longest signals (up to 20 ms) occur in the initial or "search" phase, and the shortest signals (<1 to 2 ms) occur during the "approach" phase (*2*). The mechanism for neural representation of sound duration is not simply one in which neurons respond throughout the sound, because most auditory neurons above the lower brainstem respond transiently (*3*).

Although duration-tuned neurons are found in the inferior colliculus of bats (*4*), present evidence is not sufficient to rule out the possibility that duration tuning is generated at an earlier stage. The inferior colliculus is an integration center that receives the parallel inputs of auditory pathways from the lower brainstem (*5*, *6*). The prominence of inhibition in the inferior colliculus (*7*) suggests that duration tuning is controlled by neural inhibitory mechanisms. However, there has been no evidence to support this idea. This report provides evidence that duration tuning is constructed in the inferior colliculus through the interaction of excitatory and

Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA.

*To whom correspondence should be addressed.

inhibitory inputs that are temporally offset from one another.

We recorded from single neurons in the inferior colliculus and in the cochlear nucleus of awake big brown bats (*8*). We systematically varied sound duration in order to determine whether a neuron responded more to some durations than to others. None of the 62 neurons sampled in the cochlear nucleus showed a preference for specific durations, but 45 of the 136 neurons in the inferior colliculus did (Fig. 1). Of these, 26 responded best to pure tones and 19 to frequency-modulated (FM) sweeps. When FM stimuli were presented, the FM depth was held constant; thus, when duration lengthened, the FM rate decreased. We cannot rule out the possibility that FM-sensitive neurons were tuned to FM rate rather than to FM duration. However, neurons that could be driven by both pure tones and FM sweeps were tuned to longer durations for FM sweeps than for tones. This observation suggests that these neurons responded to the duration of their best excitatory frequency within the sweep. Further studies that systematically vary FM rate, depth, and duration would be necessary to determine whether the response is to FM rate or to duration of a frequency component in the FM sounds. We include the data on these neurons (Fig. 1B) because they are tuned to one or more time-varying parameters of FM sounds, and this tuning is constructed in the inferior colliculus.

The "best duration"—the duration to which a neuron responded with the maximum number of spikes—was usually within the range of durations found in echolocation sounds; the exceptionally long best durations were within the duration of communication sounds (*9*). All duration-tuned neurons responded transiently with one or a few spikes. Multiple spikes per stimulus usually occurred only at the best duration. For neurons tested at different sound intensities, duration tuning shifted only slightly, usually to a shorter duration. All duration-tuned neurons were located in the caudal half of the inferior colliculus. For neurons that responded to pure tones, the range of best frequencies was 23 to 64 kHz; they were not sharply tuned to frequency (*6*). The absence of duration-tuned neurons in the cochlear nucleus, together with the observation that we have not found duration-tuned neurons in the nuclei of the lateral lemniscus or superior olives (*10*), support the idea that duration tuning is a property of the inferior colliculus.

To investigate further, we applied antagonists of γ-aminobutyric acid (GABA) or of glycine, two inhibitory transmitters used in the inferior colliculus (*7*). Twenty-one duration-tuned cells were tested with blockers of GABA or glycine or both. The duration tuning of 16 of these cells was altered by the blockage of inhibition (*11*). Two examples are shown (Fig. 2). For both neurons, application of bicuculline, a GABA antagonist, eliminated duration tuning. Application of strychnine, a glycine antagonist, almost completely eliminated duration tuning; there was still a slightly stronger response to the control best duration. The fact that application of either antagonist eliminated or greatly reduced duration tuning suggests that both GABA- and glycine-containing inputs contribute to this tuning.

Preliminary observations made with whole-cell patch-clamp techniques (*12*) suggested that duration tuning is generated by the temporal dynamics of excitatory and inhibitory inputs to cells in the inferior colliculus. A cell's initial response to sound was always an outward current. For a cell tuned between 10 and 20 ms, at stimulus offset, there was a large inward current that was greatest for short sound durations (Fig. 3). Because the space clamp was incomplete, the cell fired action potentials and frequently fired several spikes in response to the 10- and 20-ms tones. It was not possible to tell whether the outward current that followed the spikes was evoked by the stimulus or was a consequence of the preceding depolarization. Recordings of response to 40-ms stimuli at 10 dB above the threshold for evoked spikes (bottom trace) and at the threshold indicated that the initial outward current lasted no more than ~12 ms (8 to 20 ms from stimulus onset), with a peak at ~12 ms. The effects of varying stimulus duration suggested that the outward current was limited by excitatory input that arrived after 12 ms.

These results suggest a model for the computation of sound duration. Inhibitory input arrives first and is sustained for the duration of the stimulus. At stimulus offset, there is a "rebound" from inhibition, which in itself is insufficient to produce a spike. Excitatory input is transient and it is delayed relative to the stimulus onset; in itself it is also insufficient to produce a spike. However, if the delayed excitation coincides with the rebound from inhibition, a spike occurs. This coincidence will depend on the duration of the stimulus (*13*). For the cell illustrated (Fig. 3), the rebound from inhibition, measured from sound offset, starts at about 8 ms, peaks at 13 ms, and is over by 18 ms. We assume that the excitation has a constant latency of about 15 ms, rises sharply to peak at 20 ms, and trails off gradually. At a sound duration of 5 ms, the rebound peaks at 18 ms, which is

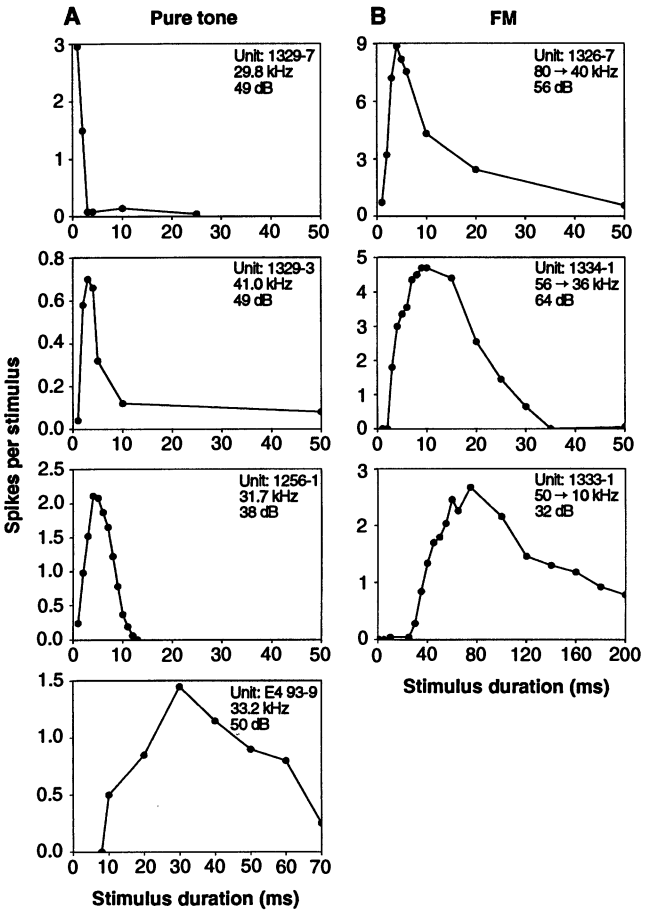

Fig. 1. Response rate (spikes per stimulus presented) as a function of duration of sound for neurons in the inferior colliculus of *E. fuscus*. (**A**) Neurons tuned to duration of fixed frequency tones. The range of best durations was from less than 1 ms (top) to about 30 ms (bottom). (**B**) Neurons tuned to duration of FM sweeps. These neurons responded only to FM sweeps, and they responded best to durations ranging from 9 ms (top) to about 75 ms (bottom). Here and in Figs. 2 and 3, the unit number, stimulus frequency, and stimulus intensity are given at the top of the figure. Unit number indicates animal (first number) and cell (second number) from which recordings were made.

within the latency of the excitatory input but before the peak of that input. At a sound duration of 10 ms, the rebound peaks at 23 ms, largely overlapping the excitatory input and producing many spikes. At a sound duration of 40 ms, the rebound starts at 48 ms, very near the tail of the excitatory input. To construct tuning to longer or shorter sounds, longer or shorter delays in the excitatory input would be required. The range of latencies of neurons in the inferior colliculus of *E. fuscus*, 30 ms or more (*14*), is adequate for the delayed inputs.

There are three important aspects of these results. First, in the same sense that there are auditory neurons tuned to specific ranges of frequency or sound location, there are neurons tuned to specific ranges of duration. As different cells have different sensitivities to frequency, different duration-tuned cells have different best durations. There is no peripheral representation of sound duration, such as place on the basilar membrane for sound frequency, place on the retina for visual space, or place on the skin for somatic space. Therefore, like tuning for sound location (*15*), tuning for duration is computed in the central nervous system. The finding that different neurons have different best durations solves a problem that arises if we assume that sound duration is represented by duration of neural response. How is a brief stimulus distinguished from a long one, if all neurons respond to both the short and the long stimulus? The results presented here resolve this issue by giving a neural place representation of sound duration. Second, results show that the computation of duration occurs in the inferior colliculus, which supports the hypothesis that the inferior colliculus integrates and transforms input from multiple sources. Recent studies have shown that inhibitory mechanisms in the inferior colliculus sharpen tuning for sound frequency or location (*16*). This study shows that tuning for a basic parameter of sound—duration (*17*)—is computed in the inferior colliculus. Finally, results indicate that the neural machinery for computing sound duration consists of three parts: (i) Inhibition arrives first and is sustained for the duration of the stimulus. (ii) Excitation is transient and delayed with respect to the stimulus onset. (iii) Duration tuning is produced by the coincidence of rebound from inhibitory inputs with delayed excitatory inputs.

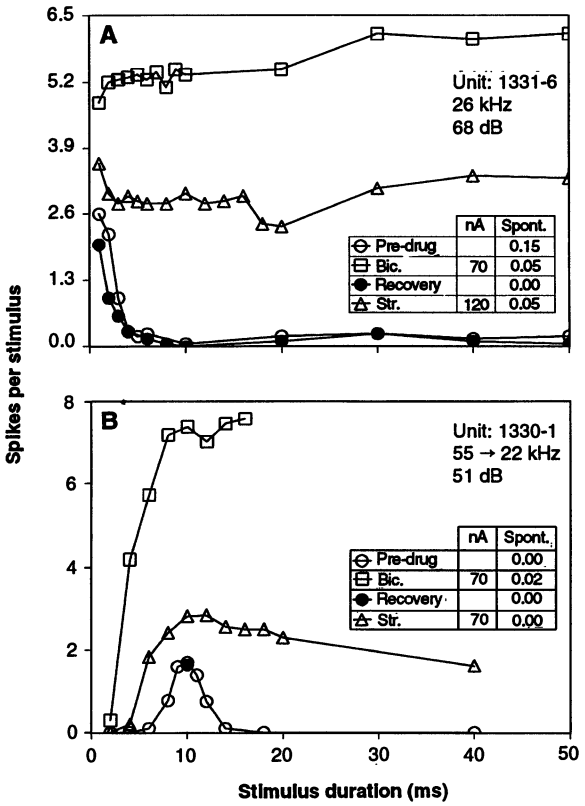

Fig. 2. Response rate of two duration-tuned neurons before and after application of antagonists to GABA and glycine. Open circles show spike counts before drug application (Pre-drug); closed circles show spike counts between and after drug applications (Recovery); open squares show spike count during bicuculline application (Bic.); open triangles show spike counts during strychnine application (Str.). The inset tables show current (in nanoamperes) used for iontophoresis and the spontaneous activity (spont.) in spikes per trial when no stimulus was presented. (**A**) A neuron that responded best to a 1-ms tone before drug application. (**B**) A neuron that responded best to downward FM sweeps of 10 ms. The spontaneous rate of activity remained essentially unchanged in both neurons with the application of either drug.

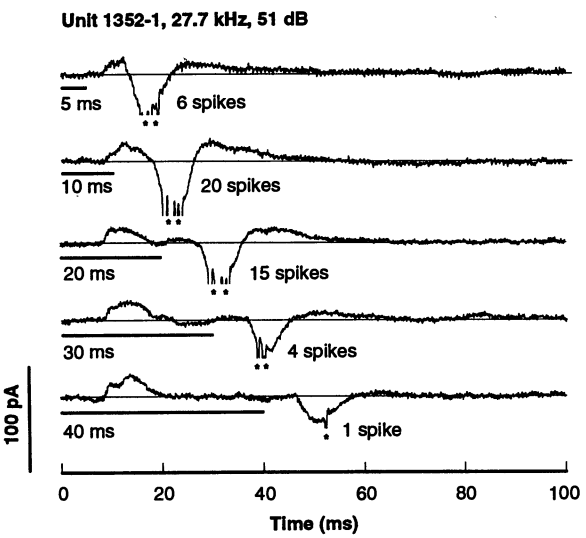

Fig. 3. Whole-cell patch-clamp recordings from a duration-tuned neuron in the inferior colliculus. The recordings were made in voltage-clamp mode so that the vertical axis is in picoamperes. On each trace, deflections above the horizontal line represent outward currents, presumably reflecting inhibitory input, and deflections below the line represent inward currents, presumably reflecting excitatory input. The stimulus duration and the number of evoked spikes are indicated under each trace. The traces are averages from eight stimulus presentations. Asterisks indicate that spikes have been truncated.

REFERENCES AND NOTES

1. B. H. Repp, A. M. Liberman, T. Eccardt, D. Pesetsky, *J. Exp. Psychol. Hum. Percept. Perform.* **4**, 621 (1978).
2. D. R. Griffin, *Listening in the Dark* (Yale Univ. Press, New Haven, CT, 1958); E. K. V. Kalko and H.-U. Schnitzler, *J. Behav. Ecol. Sociobiol.* **33**, 415 (1993); H.-U. Schnitzler and O. W. Henson Jr., in *Animal Sonar Systems*, R. G. Busnel and J. F. Fish, Eds. (Plenum, New York, 1980), pp. 109–181; J. A. Simmons and A. D. Grinnell, in *Animal Sonar: Processes and Performance*, P. E. Nachtigall and P. W. B. Moore, Eds. (Plenum, New York, 1988), pp. 353–385; J. A. Simmons, *Cognition* **33**, 155 (1989); ———, M. B. Fenton, M. J. O'Farrell, *Science* **203**, 16 (1979).
3. G. D. Pollak and J. H. Casseday, *The Neural Basis of Echolocation in Bats* (Springer-Verlag, Berlin, 1989).
4. Neurons that are tuned for sound duration were first seen in the frog's midbrain [H. D. Potter, *J. Neurophysiol.* **28**, 1155 (1965); D. M. Gooler and A. S. Feng, *ibid.* **67**, 1 (1992); for review, see A. S. Feng, J. C. Hall, D. M. Gooler, *Prog. Neurobiol. N.Y.* **34**, 313 (1990)]. In the inferior colliculus of the big brown bat, some neurons respond best to specific combinations of sound duration and repetition rate [A. D. Pinheiro, M. Wu, P. H.-S. Jen, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **169**, 69 (1991)]. In our study, we used a slow repetition rate to demonstrate that duration tuning occurs even under conditions in which the neuron has sufficient time between stimuli for recovery to full responsiveness.
5. I. R. Schwartz, in *Handbook of Auditory Research*, vol. 1, *The Mammalian Auditory Pathway: Neuroanatomy*, A. N. Popper and R. R. Fay, Eds. (Springer-Verlag, New York, 1992), pp. 117–167.
6. J. H. Casseday and E. Covey, *J. Comp. Neurol.* **319**, 34 (1992).
7. R. C. Roberts and C. E. Ribak, *ibid.* **258**, 267 (1987); M. Vater, M. Kössl, A. K. E. Horn, *ibid.* **385**, 183 (1992); P. P. Edgar and R. D. Schwartz, *J. Neurosci.* **10**, 603 (1990).

8. Methods of surgery and extracellular recording were the same as those described previously (*6, 10*) and were conducted in accordance with institutional guidelines. In anesthetized bats, a metal post was attached to the skull, and a small hole was made in the skull overlying the inferior colliculus. After recovery from anesthesia, the bat was placed in a comfortable restraining device, and the post was secured to a stereotactic device. All physiological recordings were made while the bat was unanesthetized. The experiment was terminated if the bat showed any signs of discomfort. All parameters of the stimuli were controlled by computer and were generated by a digital-to-analog converter. The rise-fall times were 0.5 or 0.25 ms. The stimuli always started at the zero crossing point of the sine wave. Stimuli were presented at a rate of three per second. While searching for neurons, stimulus frequency and duration were varied; pure tones, FM sweeps, and noises were used. Twenty, 50, or 100 trials were presented at each stimulus duration tested, spike response times were digitized, and spike counts were recorded. To administer blockers of inhibitory transmitters, we glued a recording electrode to a five-barrel micropipette so that the tip of the recording electrode extended about 10 μm from the tip of the micropipette [for detailed description, see D. C. Havey and D. M. Caspary, *Electroencephalogr. Clin. Neurophysiol.* **48**, 249 (1980)]. Barrels were filled with 500 mM GABA (pH 3.5 to 4.0), 500 mM glycine (pH 3.5 to 4.0), 20 mM bicuculline methiodide (pH 3.0), or 20 mM strychnine HCl (pH 3.0 to 3.5). After collecting control data, we administered bicuculline or strychnine by iontophoresis (20 to 120 nA), the cell was tested again at the same stimulus durations, administration of the drug was terminated, the cell was tested for recovery at some sound durations, and the drug not administered in the first test was applied.
9. The best duration ranged from 1 to 30 ms for neurons that responded best to pure tones; most (20 out of 26) were tuned to durations of 5 ms or less. The best duration for neurons that responded best to FM sweeps ranged from 3 to 75 ms; most (13 out of 19) were tuned to durations between 3 and 8 ms. Although some best durations were longer than the longest echolocation sounds, they were still within the range of communication sounds. Isolation calls of infant *Eptesicus* can be over 100 ms in duration [E. Gould, *Commun. Behav. Biol. Part A Orig. Artic.* **5**, 263 (1971)].
10. E. Covey and J. H. Casseday, *J. Neurosci.* **11**, 3456 (1991); E. Covey, *J. Neurophysiol.* **69**, 842 (1993); J. H. Casseday, E. Covey, M. Vater, *J. Comp. Neurol.* **278**, 313 (1988); E. Covey, M. Vater, J. H. Casseday, *J. Neurophysiol.* **66**, 1080 (1991).
11. In three of the five neurons that were unaffected, we were able to test only the effects of blocking one inhibitory transmitter; thus, we cannot rule out the possibility that duration tuning would have been altered by application of the other drug. The duration tuning of the other two neurons was not affected by either drug. At this point, we do not know whether these two unaffected neurons reflect a technical problem in drug delivery or a real population of neurons that receive input from other neurons that are already tuned to duration.
12. Intracellular patch-clamp recordings were made from awake animals after a procedure similar to that described for in vivo recordings in the cortex of anesthetized animals [B. Jagadeesh, C. M. Gray, D. Ferster, *Science* **257**, 552 (1992)]. Animals were prepared as described (*8*). Electrode resistances ranged from 5 to 8 megohm. Electrodes were filled with a solution of 110 mM cesium gluconate, 11 mM EGTA, 10 mM $CsCl_2$, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Hepes, 2 mM adenosine triphosphate, and 0.3 mM guanosine triphosphate. The pH was adjusted to 7.2; osmolarity was adjusted to 280 mosmol [E. Covey, B. R. Johnson, D. Ehrlich, J. H. Casseday, *Soc. Neurosci. Abstr.* **19**, 535 (1993)].
13. Similar theoretical models have been proposed for neurons tuned to pulse-echo delay in bats [N. Suga, *Neural Networks* **3**, 3 (1990)].
14. S. Haplea, E. Covey, J. H. Casseday, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.*, in press.
15. J. H. Casseday and E. Covey, in *Directional Hearing*, W. Yost and G. Gourevitch, Eds. (Springer-Verlag, Berlin, 1987), pp. 109–145; S. Kuwada and T. C. T. Yin, *ibid.*, pp. 146–176; M. Konishi, *Sci. Am.* **268**, 66 (Apr 1993).
16. C. F. Faingold, C. A. Boersma-Anderson, D. M. Caspary, *Hear. Res.* **52**, 201 (1991); L. Yang, G. D. Pollak, C. Resler, *J. Neurophysiol.* **68**, 1760 (1992); T. J. Park and G. D. Pollak, *J. Neurosci.* **13**, 2050 (1993); M. Vater, H. Habbicht, M. Kössl, B. Grothe, *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **171**, 541 (1992).
17. R. B. Masterton has argued that brief sounds have a special biological importance for all mammals [*Trends Neurosci.* **15**, 280 (1992)]. Our results provide a neural definition of "brief."
18. We thank D. Caspary for instruction on iontophoretic drug application; J. A. Kauer for instruction on whole-cell patch-clamp recording; and B. Rosemond, C. Lucius, and S. Keros for technical assistance. B. R. Johnson, during the course of experiments on another topic (thesis, Duke University, Durham, NC, 1993), deserves credit for first finding a cell whose duration tuning was affected by antagonists of GABA or glycine. Supported by grants from NIH (DC-00287 and DC-00607) and NSF (IBN-9210299).

15 December 1993; accepted 22 March 1994

Differential Effects of Apolipoproteins E3 and E4 on Neuronal Growth in Vitro

B. P. Nathan, S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley, R. E. Pitas*

Apolipoprotein E4 (apoE4), one of the three common isoforms of apoE, has been implicated in Alzheimer's disease. The effects of apoE on neuronal growth were determined in cultures of dorsal root ganglion neurons. In the presence of β-migrating very low density lipoproteins (β-VLDL), apoE3 increased neurite outgrowth, whereas apoE4 decreased outgrowth. The effects of apoE3 or apoE4 in the presence of β-VLDL were prevented by incubation with a monoclonal antibody to apoE or by reductive methylation of apoE, both of which block the ability of apoE to interact with lipoprotein receptors. The data suggest that receptor-mediated binding or internalization (or both) of apoE-enriched β-VLDL leads to isoform-specific differences in interactions with cellular proteins that affect neurite outgrowth.

Apolipoprotein E (apoE) is a 34-kD protein that mediates the binding of lipoproteins to the low density lipoprotein (LDL) receptor and to the LDL receptor–related protein (LRP) (*1, 2*). In the nervous system, apoE is synthesized and secreted primarily by astrocytes (*3, 4*) and macrophages (*5*). It is a major apolipoprotein in the brain (*6*) and in cerebrospinal fluid (*7*). Apart from its role in maintaining plasma cholesterol homeostasis, apoE is thought to participate in the mobilization and redistribution of lipids during normal development of the nervous system (*4*) and in the regeneration of peripheral nerves after injury (*8*). Three major isoforms of apoE (apoE2, apoE3, and apoE4) are products of three alleles (ε2, ε3, and ε4, respectively) at a single gene locus on chromosome 19 (*9*). The most common isoform is apoE3, which contains cysteine and arginine at positions 112 and 158, respectively. Both positions contain cysteine in apoE2 and arginine in apoE4.

The ε4 allele is specifically associated with both familial late-onset and sporadic Alzheimer's disease (AD), and the gene dose of ε4 is a major risk factor for the disease (*10*). Accumulated apoE is found in the senile plaques and neurofibrillary tangles seen in postmortem examinations of the brains of AD patients (*11*). Alzheimer's disease patients who are homozygous for the ε4 allele exhibit more highly developed senile plaques at autopsy than other AD patients (*12*). Thus, apoE4 may contribute to the pathogenesis of AD. Because neuronal pathology is a characteristic of AD, we examined the effect of human apoE on neurite outgrowth. Here we demonstrate that human apoE4 and apoE3 have different effects on the outgrowth of neurites from dorsal root ganglion (DRG) neurons in culture.

Rabbit DRG neurons were used as a model system to study the effects of lipids and apoE on neurite outgrowth. Previously, rabbit β-migrating very low density lipoproteins (β-VLDL), which are cholesterol-rich lipoproteins, have been shown to stimulate

B. P. Nathan, S. Bellosta, D. A. Sanan, Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, University of California, San Francisco, Post Office Box 419100, San Francisco, CA 94141–9100, USA.
K. H. Weisgraber and R. E. Pitas, Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, and Department of Pathology, University of California, San Francisco.
R. W. Mahley, Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Department of Pathology, and Department of Medicine, University of California, San Francisco.
*To whom correspondence should be addressed.