following exception: We defined the "signal" in each section by taking a measurement from a 75 by 75 μm area within the SCN and subtracting a background measurement from an area of equal size lateral to the SCN.

27. The AP-1 consensus oligonucleotides used in binding assays were oligo 1 (Oncogene Science), double-stranded 36-nucleotide, 5′-GATCCATCG**TGACTCA**GCGATCTCG**TGACTCA**GCGG-3′; oligo 2 (Promega), double-stranded 21-nucleotide, 5′-CGCTTGA**TGAGTCA**GCCGGAA-3′. (Boldface indicates consensus AP-1 binding sites.) Oligonucleotides were radiolabeled with [α-^{32}P]deoxycytidine 5′-triphosphate and terminal deoxynucleotidyl transferase and gel-purified by electrophoresis on a 15% polyacrylamide gel. Whole-cell extracts of brain tissue were prepared by sonication of each SCN sample (*17*) at 4°C in 50 μl of buffer [20 mM Hepes (pH 7.8), 125 mM NaCl, 5 mM $MgCl_2$, 12% glycerol, 0.2 mM EDTA, bovine serum albumin (BSA) (1 mg/ml), 0.1% Nonidet P-40, 5 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride, leupeptin (0.5 μg/ml), pepstatin (0.7 μg/ml), aprotinin (1 μg/ml), and bestatin (40 μg/ml)]. Extracts were then centrifuged at 15,600*g* for 10 min (at 4°C) and the supernatants collected. Binding assays were performed with 10 μl of SCN cell extract, 1.5 μg of poly[d(I-C)], 5 μl of buffer [50 mM Hepes (pH 7.8), 5 mM spermidine, 15 mM $MgCl_2$, 36% glycerol, BSA (3 mg/ml), 0.3% Nonidet P-40, and 15 mM DTT], and 30,000 cpm of ^{32}P-labeled oligonucleotide, with water added to a final volume of 25 μl. Reactions were incubated for 15 min on ice before addition of ^{32}P-labeled oligonucleotide, then for an additional 15 min at 22°C. When competition with unlabeled oligonucleotide was performed, a 50-fold molar excess of oligonucleotide relative to the radiolabeled probe was added to the binding assay. When antibodies were included, 1 μl was added to the reaction 15 min before the addition of the probe. The antibody to Fos is a rabbit antiserum made against the synthetic peptide corresponding to amino acids 127 to 156 of mouse Fos; rabbit antiserum against bovine neuron-specific enolase (Accurate) was used as a control antibody. Samples were subjected to electrophoresis on 4% nondenaturing polyacrylamide gels, dried, and autoradiographed.

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Technical Comments

Ultrasonic Hearing

M. L. Lenhardt *et al.* (*1*) suggest that "bone-conducted, ultrasonic stimulation may provide an alternative therapeutic approach for the rehabilitation of severe hearing loss." They argue that when speech signals are used to modulate the amplitude of an ultrasonic carrier, people detect and recognize speech sounds by physiological mechanisms other than those that normally transduce audible speech.

There is an alternative explanation for the findings of Lenhardt *et al.* that is consistent with classic auditory physiology, but does not support the possibility of using this approach to bypass a damaged cochlea. Even a slight, even-order (rectifying) nonlinearity in the transfer path from transducer to skull would result in demodulation. The speech signals, which had been modulated onto the ultrasonic carrier, could be converted back into audio frequency bone-conducted stimuli, capable of being transduced by conventional cochlear processes.

The stimuli used by Lenhardt *et al.* were intense compared with those used for bone conduction testing in the audio frequency range. Lenhardt *et al.* specify their stimuli as acceleration, in decibels with reference to 10^{-3} m/sec^2, whereas the American National Standards Institute S3.26-1981 standard (*2*) for zero hearing level for the B-71 bone vibrator averages 30.5 dB with reference to 1-μN force at 2 to 4 kHz. If this force is applied to a 5-kg head moving as a rigid body, an acceleration of 6.7×10^{-6} m/sec^2, or 43 dB below Lenhardt's reference level (*1*), would result. Response thresholds to ultrasound are reported (*1*) at +82 to +112 dB (with reference to 1×10^{-3} m/sec^2) or 125 to 155 dB above threshold accelerations at audio frequencies. If only 10% of the head mass were to effectively move in response to bone-conducted ultrasound, their thresholds would still be 105 to 135 dB more intense than audio-frequency thresholds.

A slight nonlinearity, resulting in demodulation of audible speech signals, might be difficult to observe in spectral analysis with only a 60-dB dynamic range. A better test would be to measure cochlear potentials. Foster and Wiederhold (*3*) showed that, in cats, pulsed ultrasound produced cochlear microphonics and compound action potentials that were indistinguishable from those produced by audible transient stimuli. We suspect that, were a study to be performed similar to (*3*) that used the stimuli presented by Lenhardt *et al.*, it would reveal that these stimuli were present in the cochlear microphonic and thus available in the audible range within the cochlea.

The results obtained by Lenhardt *et al.* in human subjects were not clearly better than could have been achieved by presenting the same speech stimuli in the normal audio range. Even their "deaf" subjects had *mean* thresholds [(*1*), figure 1A] that were within about 55 dB of the normal threshold at 250 Hz [we assume that the abscissa of figure 1A in (*1*) should have been labeled from 0 to 10,000 Hz for the air conduction thresholds, and that the lowest frequency tested was about 250 Hz]. Thus, some of these subjects could probably discriminate some speech stimuli when presented with adequate stimulus intensity. Two of the nine "deaf" subjects displayed accuracy scores of 20 and 30%, respectively, on a closed-set test for which random performance would produce a score of 16%. Even if these two performances had been significantly better than chance, presentation of the same speech materials as high-intensity audio stimuli might have given similar results.

One critical control experiment would be quite simple. If speech signals were reaching the cochlea as audio-range signals after demodulation, they would be maskable by audio-range noise maskers.

Until more conventional mechanisms can be excluded, it appears premature to suggest a separate ultrasonic receptor, particularly when the structure suggested to detect ultrasound, the saccule, responds to vibrations from zero to only 2 kHz in the squirrel monkey (*4*).

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Response: Dobie and Wiederhold postulate that the relatively intense ultrasonic signals used in our study (*1*) could allow demodulation of ultrasonic speech into the audio frequency range. The normal hearing ear is an excellent demodulation detector. With modulation, all listeners in our study reported hearing the sidebands and the carrier, which would have been impossible had the signals been demodulated. With deaf subjects we again found no evidence for

demodulation. The three deaf subjects had pure tone hearing averages (0.5, 1.0, and 2.0 kHz) at a 90-dB hearing level in their better ears, with thresholds at 250 Hz that ranged from a 95- to 115-dB sound pressure level. (The abscissa in figure 1A of (*1*) should have been labeled from 2,000 to 10,000 Hz). Thus, they had little audio sensitivity for detecting, let alone discriminating, the filtered speech (300-Hz high pass) signals that were modulated. Furthermore, when the carrier and lower sideband were suppressed (they were audible and this interfered with intelligibility of the stimulus), all deaf subjects scored 40% correct on a test of closed set speech discrimination (*1*), a result Dobie and Wiederhold do not mention. Discrimination by deaf subjects was significantly ($P < 0.001$) above chance (16%) and was comparable to discrimination by subjects with cochlear implants (*2*).

Dobie and Wiederhold suggest that these results were not better than one could obtain by presenting the same speech material in the auditory range. This does not account for the severity of the hearing impairment experienced by the deaf or for the failure of amplification to help these individuals. Our subjects wore power hearing aids with outputs near 130-dB sound pressure level. Their performance on the same speech test with amplification never reached chance levels.

Dobie and Wiederhold suggest two alternative paradigms. One, used by Foster and Wiederhold (*3*) to determine if auditory responses could be produced by pulsed ultrasound in cats, is not directly comparable to ours. The stimuli used by Foster and Wiederhold were intense (~30 W/cm^2), focused, transient (30 to 60 μs), 5-mHz signals delivered directly to the brain; our signals were continuous, diffuse, and under 0.05 mHz. In the cochlear microphonic frequency spectrum, half of the six cats were found to have a dominant low audio frequency. If ultrasound induces fundamental skull resonance, which in turn induces audio frequency stimulation of the cochlea, then all cats should have exhibited a dominant low audio frequency. All cats had high frequencies present in the cochlear microphonic.

We have now pulsed (60 μs) the 30-kHz signals used in (*1*), and as energy spread down into the audio range (because a transient was added to the ultrasonic tone), we observed a well-known physical effect of abrupt stimulus gating, an effect that we carefully avoided in (*1*).

A second alternative paradigm argues that, in the presence of forced audio frequency skull resonance [1.8 kHz in humans (*4*)], demodulation is possible. If so, speech spectrum noise should mask the ultrasonic stimuli. Masking of ultrasound was not possible with audio speech spectrum noise (0.3 to 4.0 kHz), which delivers up to 3*g* root mean square of acceleration, whereas noise in the ultrasonic range was an effective masker. We have now recorded evoked responses to ultrasonic tone bursts with simultaneous speech spectrum noise; no change in evoked wave latency was observed, and both the masker and tone were audible. The inability of intense audio noise to mask ultrasound supports the premise that some extra-cochlear mechanism is present.

We are criticized by Dobie and Wiederhold for speculating that the saccule is an ultrasonic organ, but freestanding hair cells with short cilia, present in the human striola region of the saccule, can be set into resonance by ultrasound from 20 to 100 kHz as a function of cilia height (*5*). Cilia bundles are distributed spatially by height, which provides a peripheral basis for ultrasonic frequency discrimination (*6*). These results do not rule out a contributory role for cochlear inner hair cells with short ciliary bundles, particularly in normal hearing persons, but they do suggest an independent role for the saccule in deafened individuals. We reiterate our conclusion (*1*) that ultrasonic speech stimulates a known receptor in a different way, with different frequencies from those in normal hearing persons. Such an explanation is parsimonious in view of accepted auditory theory and could provide an alternative approach to aural rehabilitation.

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