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17. Dendritic field outlines were drawn at a magnification of 278 by the camera lucida technique, and dendritic field areas were determined by planimetry.
18. Dendritic spines, in the same focal plane on dendritic shafts, were counted under oil immersion at  $\times 2000$  magnification. A minimum of ten spine counts at least  $10\ \mu\text{m}$  long, distributed randomly over proximal and distal dendrites, were made for each cell. Ten cells per animal were used.
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## Holographic Assessment of a Hypothesized Microwave Hearing Mechanism

**Abstract.** *Exposure of the head to pulse-modulated microwaves induces the perception of a sound. It has been hypothesized that the electromagnetic energy is converted to acoustic energy in the skull and then conducted through the bone. Dynamic time-averaged interferometric holography showed that the predicted motion of head tissue did not occur. An alternative locus for this hearing effect is suggested.*

A person exposed to pulse-modulated microwave energy can perceive the effect as a sound, such as a buzz (1-3). The mechanism for the perception is unknown, although it does not appear to be located in the brain itself (4).

Foster and Finch (5) and Chou *et al.* (6) concluded that the electromagnetic energy is transduced into acoustic energy by thermoacoustic expansion in the muscle or bone of the head. In the mechanism they suggested, thermoacoustic expansion would generate acoustic waves that would be conveyed via bone conduction to the tympanic membrane and middle ear. There are many mechanisms of bone conduction (7), and the one involved in this process was not specified.

The thermoacoustic expansion-bone conduction hypothesis is based on a study of acoustic transients induced by the impingement of microwave pulses on water in a tank, with the water representing an approximation of the head, and a hydrophone an approximation of the middle ear and cochlea. Support for this hypothesis came primarily from inferences drawn from studies of cochlear microphonics (6, 8).

The thermoacoustic expansion-bone conduction hypothesis is attractive for its apparent simplicity and because it is based on a well-known physical phenomenon (9). However, when one considers that ordinary auditory perception is not fully understood and that there are multiple mechanisms for bone conduction (7), one finds reason to question this hypothesis. What is needed is a direct physiological test that would show whether or not the skull, or the soft tissue of the head of a mammal, actually shows an acoustic wave when exposed to pulses of electromagnetic energy.

In the experiment described here we attempted, by use of dynamic time-averaged interferometric holography, to find the predicted motion in hair, skin, muscle, bone, and brain in the rat and the guinea pig. This well-established nondestructive testing technique is commonly used in the study of acoustic waves in material. Double-pulse holography was not used since it requires the use of high-power laser pulses that in themselves can induce an acoustic wave in the head and be a confounding variable (10).

The time-averaged holography technique consists of making a single ho-

lographic recording of an object in which vibratory motion has been induced. It requires that the exposure time in recording the hologram be long compared to one period of the vibration cycle. The hologram effectively stores an array of data representing the time average of all positions of the vibrating object. Where the displacement of the object is zero, the reconstructed image intensity will be brightest. Areas of the object that move will be dim or black in the holographic image. The technique provides information about the amplitude of vibration and the location of vibratory nodes, and is applicable to nonsinusoidal motions. The sensitivity to motion is  $0.06\ \mu\text{m}$ . Von Békésy's data (11) concerning vibration of the head in a sound field and its role in hearing by bone conduction, and the analysis by Naftalin (12) of hearing thresholds, indicate that the technique would be sensitive to the hypothesized motion if it existed.

The animal was placed on its abdomen on a surface of Eccosorb FR-340 microwave energy absorber placed on the surface of a plywood table that was designed to be isolated from vibration. In some tests, the microwave energy absorber was replaced with a cement block. The microwave energy was generated by a pulsed triode source and directed, by means of a horn antenna mounted over the table, toward the surface of the table. Energy densities were measured before and after each session at the location of the animal's head with a half-wave dipole antenna (13).

An etalon-equipped krypton ion laser was used in a standard off-axis holography setup. The exposures of the Kodak S0253 film were controlled with an automatic shutter, and the films from each run were developed together. The coded sets of holograms were assessed blindly by two experimenters using both a Holographics Corporation model 1020 viewer and the diverged beam of a HeNe laser. The holographic setup was tested in several ways to verify that our technique was comparable to that used by others in nondestructive testing of materials (14).

Two series of physiological tests were run, one with guinea pigs and the other with rats. A treatment-by-subject design was used in which each animal was its own control. Because of the sensitivity of the holographic technique, all subjects were injected intraperitoneally with an overdose of sodium pentobarbital and the hair over the area of interest was clipped short and a depilatory applied. Experimentation started as soon as there was no detectable heartbeat or respira-

tion. For each animal, a set of 30 holograms was made. Half of these were made during microwave energy exposure and half were made during sham exposure in which all equipment was turned on but the power was not turned up.

Six film exposures were made of the animal's head area with the hair removed from the dorsal surface of the head and the vicinity of the left pinna. Thus, bare skin as well as hair could be visualized. Three of the time-averaged holograms were made during sham exposure and three were made during exposure to microwave energy. Exposure and sham exposure were alternated. The skin on the dorsal surface of the head and the pinna was removed and six holograms were made of the musculature. The muscle tissue was removed from the dorsal surface of the head and mastoid area and six holograms were made of the skull. The dorsal surface of the skull was removed and six holograms were made of the brain. The last six holograms were made after the brain was removed from the skull and the base of the cavity (the dorsal surface of a bulla) could be holographed.

In experiment 1, ten Sprague-Dawley rats (350 to 400 g) were used. The microwave energy carrier frequency was 1.275 GHz, the pulse width 25  $\mu$ sec, and the pulse repetition rate 50 pulses per second. The incident peak power was 1700 mW/cm<sup>2</sup>. In five of the animals, an additional set of holograms was made at 100 pps as each layer of tissue was removed, starting with the holograms made of the muscle tissue. To check for possible artifacts caused by the bone and brain not being tested immediately after death, two additional animals were used. The skull was exposed during anesthesia and the bone and brain were tested immediately after death.

In experiment 2, we used 16 adult male guinea pigs, eight of them being tested at a frequency of 1.1 GHz in a 2 by 2 by 2 factor design. The factors were peak power density at 1250 and 8500 mW/cm<sup>2</sup>; pulse width at 10 and 20  $\mu$ sec; and pulse repetition rate at 25 and 50 pulses per second. Another eight animals were used at a frequency of 1.2 GHz. These are frequencies that have been found to be in the optimal range for inducing the radio-frequency (rf) hearing effect.

When we developed and viewed the holograms for the first three subjects at the 8500 mW/cm<sup>2</sup> power level we found that the microwave energy absorber on which the animal was resting was being moved by the microwave energy pulses; this was understandable in view of the

work of Sharp *et al.* (15). We therefore tested the remaining animals at the highest power level on a cement block that did not significantly perturb the microwave field. We also checked for possible artifacts as in experiment 1.

The holographic images of each animal during microwave energy exposure were coded and compared on a blind basis with the images of the same animal during sham exposure for the two experiments. There were no detectable differences in any of the tissues for any animal.

Several lines of evidence suggest that the thermoacoustic expansion-bone conduction hypothesis is of doubtful validity. The inferences drawn from cochlear microphonic data and used to support the hypothesis can also be questioned. First, in the direct physiological tests reported here we did not find the predicted motion. Second, patterns of glucose consumption in the brains of rats exposed to pulsed microwave energy were measured by Wilson *et al.* (16) using autoradiographic techniques. In each animal, one middle ear was ablated in order to cause an imbalance in the activity of the two sides of the auditory system under conditions of sound stimulation. By comparing patterns of glucose utilization during exposure to acoustic stimuli with such patterns obtained during exposure to microwave energy, these workers were able to show that pulsed microwave energy can elicit a metabolic response in the auditory system by some mechanism other than conduction of sound through the middle ear.

Third, by means of recordings from the eighth nerve, Lebovitz and Seaman (17) found that there is a decreased microwave energy sensitivity of high-frequency auditory nerve units. But they pointed out that the thermoacoustic expansion-bone conduction hypothesis implies the presence of a pronounced high-frequency mechanical component in the microwave energy response.

Fourth, Tyazhelov *et al.* (18) determined the loudness of rf sound as a function of pulse width and repetition rate and studied beat phenomena. They concluded that the thermoacoustic expansion-bone conduction hypothesis was not supported by their data at the low power levels suitable for rf hearing.

Fifth, Frey and Eichert (19) with the aid of musicians matched acoustic energy to the rf sound induced by microwave energy. The microwave signals used would have been expected to induce the repetition pitch phenomena if the hypothesis were valid. The predicted repetition pitch did not result. This suggests

that the energy is not transduced into acoustic energy before reaching the cochlea. Further, it suggests that the mechanism does not involve in toto the cochlear mechanisms of hearing.

Only the experiments of Chou and co-workers (6, 8) are interpreted as supporting the hypothesis. Chou *et al.* (8) recorded cochlear microphonics from the round window of guinea pigs exposed to microwave energy. Though they selected their subjects on the basis of the animal giving a large cochlear microphonic ( $> 0.5$  mV) in response to acoustic energy, the cochlear microphonic in response to microwave exposure was obtained by averaging, because it was quite small, less than 50  $\mu$ V. They used peak powers "up to 10,000 watts" with the animal's head in a circular waveguide and "more than 99% of the microwave energy was absorbed by the [guinea pig's] head." The microwave generation equipment was in the same test room as the subject, so that the sound level in the vicinity of the animal's head was about 65 dB. In a later study, Chou *et al.* (6) included a microwave frequency of 918 MHz as well as 2450 MHz, used cats as well as guinea pigs, and used a horn applicator in close proximity to the head, as well as a cylindrical waveguide. They did not use free-field exposures at incident peak powers of approximately 1 W/cm<sup>2</sup> as was done when rf hearing was defined. Although they interpreted their data as supporting the thermoacoustic expansion-bone conduction hypothesis, other interpretations are possible.

Straub (20), for example, suggested the Ludwig-Soret effect as an alternative interpretation for the data of Chou *et al.* This effect involves thermally induced electric fields set up in ionic liquids. Straub suggested that the changes in the electric field in a membrane that are brought about by a large thermal gradient might be large enough to cause depolarization or movement of calcium from the surface of the membrane.

Another possible interpretation of the data of Chou *et al.* is that the microphonics are a response to acoustic pulses emitted by their signal source or generated in their waveguide or horn applicator. Acoustic clicks correlated with the intensity of the microwave energy pulses emitted were observed by one of us (A.H.F.) using the same type of equipment as Chou *et al.* Because of the microphonic's latency from the microwave pulse artifact and because they "detuned the head," Chou *et al.* did not believe their microphonic was from acoustic energy from the generator. But they assumed that the correlated acous-

tic pulse was emitted from their equipment at the instant that the microwave pulse was emitted, which is not necessarily the case.

Many materials, including such screening as that in the circular waveguide used by Chou *et al.*, "sing." In early rf hearing work, A.H.F. had difficulty carrying out tests in which the subject's head was to be shielded, because so many materials and material junctions "sing" acoustically in response to and in correlation with high peak pulsed microwave energy. The inside of horn antennas also often "sing" very softly. Chou *et al.* (6) report that when using a horn applicator "it was necessary to place a cat's head in close proximity [to the horn aperture] in order to record a detectable CM [cochlear microphonic]." When we consider that microphonics are found only after averaging from selected subjects, and then only in a waveguide or with a horn applicator when it is very close to the head, it is difficult to dismiss the possibility of response to extraneous acoustic energy in the experiments of Chou *et al.*

We also note that Chou and Guy (21) show a picture of an animal with its head in the circular waveguide. The animal's head is lying on what they state is a slab of polystyrene foam. They also report that they used a carbon electrode placed against the round window to record the microphonics. In our experiment 2, we had to replace the microwave absorber (polystyrene foam block with carbon deposited in cone-shaped depressions on the underside) because the holographic technique showed that the polystyrene foam block vibrated in response to high peak power microwave pulses.

The evidence discussed herein suggests that the locus of the rf hearing effect is in the cochlea. Among the many mechanisms in the cochlea that might account for the perception of microwave energy, consideration should be given to the possibility of thermoacoustic expansion within the cochlea. White (22) suggested that transient elastic waves may be generated by thermoacoustic expansion in cochlear structures and thus induce the rf hearing effect.

If the locus of the effect is within the cochlea, as it appears, then defining the mechanism will be difficult. But the microwave-induced hearing phenomenon may prove useful in the analysis of the function of a portion of the auditory system that is poorly understood.

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## Human Chorionic Gonadotropin: Induction of Ovulation in the Squirrel Monkey

**Abstract.** *The minimum dose of human chorionic gonadotropin that would cause ovulation in the squirrel monkey (*Saimiri sciureus*) was found to be between 100 and 250 international units.*

For many years human chorionic gonadotropin (hCG) has been the drug of choice for inducing ovulation in humans and in domestic and laboratory animals. Dosages used for humans have been reported to be as high as 20,000 I.U. of hCG administered as a single dose, but more generally doses of 2000 to 8000 I.U. are used (1). For ovulation induction in domestic animals the dosage range is 500 to 5000 I.U. of hCG. The minimum effective dose (MED) that causes ovulation in primates is not known. In the rabbit, the MED is 25 I.U. (2), and administration of only 100 I.U. of hCG has been reported to cause chromosome abnormalities in 6-day blastocysts (3).

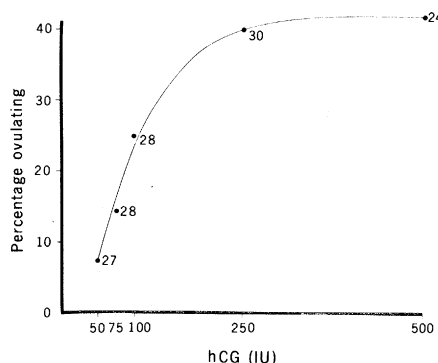


Fig. 1. Percentage of squirrel monkeys ovulating in response to a given dose of hCG after treatment with FSH for 4 days. Values at each point indicate numbers of animals tested.

Human chorionic gonadotropin is commonly used to treat human obesity (in doses of 1500 to 2000 I.U. administered daily over extended periods) despite warnings from the Food and Drug Administration that hCG has not been demonstrated to be effective adjunctive therapy in weight control programs (4). In view of the concern about excessive administration of hCG to humans, the present study was designed to determine the minimum dose of hCG that would induce ovulation in a nonhuman primate, the squirrel monkey (*Saimiri sciureus*).

Squirrel monkeys ( $N = 137$ ) of Bolivian and Guyanese origin (5) were subjected to a standard regimen of follicle-stimulating hormone (FSH) administration (6) for 4 days (1 mg of FSH per day). On the afternoon of the fourth day a single injection of 50, 75, 100, 250, or 500 I.U. of hCG (A.P.L.® Ayerst, Montreal) was given intramuscularly. The ovaries were examined by means of a laparoscope (7) 36 hours later to determine if ovulation had occurred. Because there is a seasonal response to ovulation in squirrel monkeys (8), all trials were conducted during April, May, and June of 1978 and 1979.

The responses of these nonhuman primates to the different doses of hCG are shown in Fig. 1. For the groups receiving 50, 75, 100, 250, and 500 I.U., the mean number of ovulations per female were,