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Threshold Ultrasonic Dosages for Structural Changes in the Mammalian Brain *

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The relationship between the acoustic intensity and the time duration of exposure, for a single pulse, necessary to produce a threshold lesion in the cat brain was studied. Focused ultrasound of 1, 3, and 4 MHz was employed with intensities ranging from 10^2 to 2×10^4 W/cm^2 with the corresponding pulse durations from 7 to 2×10^{-4} sec, respectively. Three types of lesions were observed attending three regions. At the lower intensities and long time durations of exposure, the lesion is produced by a thermal mechanism. At the highest intensities and shortest time durations, cavitation is believed to be the mechanism responsible for the sometimes randomly appearing lesions. At intermediate dosages, the lesions are formed by a mechanical mechanism which is thus far not well understood. These results exhibit good agreement with that of other investigators on both the cat and the rat brain.

The use of intense focused ultrasound to produce changes in the mammalian central nervous system has been described in detail by several investigators.[1–5] Characteristically, it has been found that: (1) functional changes occur instantaneously,[6] while histological changes require approximately 10 min after exposure for the first suggestions of lesion formation to appear[7]; (2) heretofore, conditions for cavitation appeared adverse and evidence for its occurrence is sufficiently lacking to discount it as a possible mechanism[6]; (3) thermal mechanisms are important in the lower dosage regions generally employed[6,8,9]; (4) the acoustic properties of white matter are significantly different from those of gray matter and these differences may be associated with vascularity[10]; and (5) direct interaction with molecular processes can be ruled out,[11] but interaction with membraneous structures may well be the site of the acoustic involvement.[12]

Though several investigators have studied ultrasonic dosage (time duration of exposure and acoustic intensity) in the low-megahertz-frequency range, the intensities employed were generally below about 10^3 W/cm^2, owing to the limitations of the available instrumentation. Thus, it was considered profitable to study the higher dosage range (to 2×10^4 W/cm^2) at 1 and 3 MHz and to examine the nature of the lesions so produced.

The irradiation technique and procedure have been described previously.[1] Briefly, the skull of the animal is removed to allow the sound to enter the brain unimpeded, though the *dura mater* is not opened. Degassed Ringer's solution is employed to couple the acoustic energy from the focusing transducer to the brain and the irradiation is performed with the brain temperature stabilized at 37°C. For the portion of the study involving cavitation levels of ultrasound, four irradiation exposures were placed in each animal, i.e., the focal region of the sound beam was, in turn, positioned 15 mm deep, with respect to the dorsal surface of the brain, 5 and 15 mm anterior, and ±5 mm lateral, with respect to ear bar zero. These positions[13] are in regions containing dense white-matter tracts and gray-matter regions in such distribution that the focal volume of the transducer included both white and gray matter. Thus, the threshold for white and gray matter could be examined in a single exposure, though this paper deals only with the threshold doses for white matter. The four single-pulse exposures were generally delivered at the same intensity, but with variable pulse durations. The animals were sacrificed 24 h after the ultrasonic irradiation and the brain was subsequently stained by both Weil and cresylecht violet and examined for histological evidence of damage.

Figure 1, the dosage curve for threshold lesions, at 1, 3, and 4 MHz plus the similar data of Pond on rat brain[8] and Lele on cat brain[3] comprises three ill-defined regions. At the lowest intensities and longest time durations of exposure, the lesions are considered to be

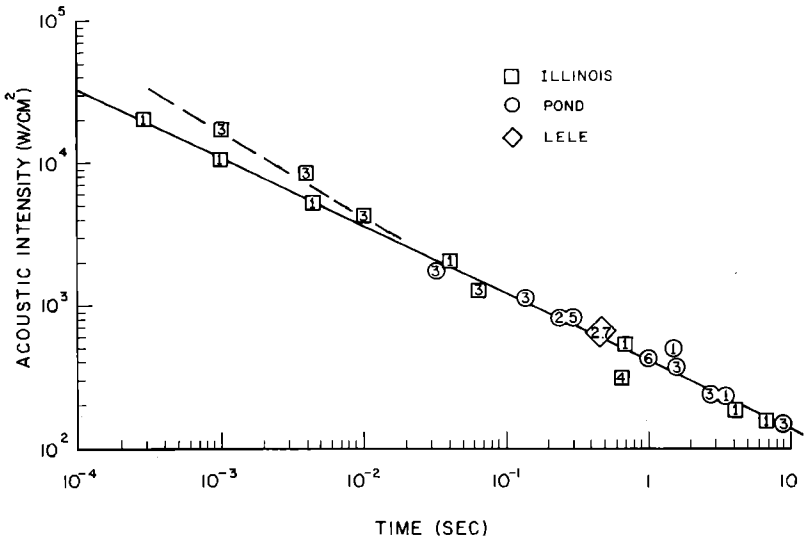

FIG. 1. Acoustic intensity versus single-pulse time duration to produce threshold lesions in white matter of the mammalian brain.

produced by heat, owing to absorption of the ultrasound in the tissue,[6] though there is not universal agreement on the upper intensity boundary.[8] In the intensity range from several hundred to approximately 1500 W/cm², thermal mechanisms do not account for the lesions. Here, the ultrasound is considered to disrupt biological structure subtly by mechanical means.[1,6] For this dosage region, the histological response to the ultrasonic irradiation has been described in great detail.[1] Briefly, white matter exhibits the lowest threshold and the lesion results in demyelination of the axis cylinders. Gray matter is more resistant and the dose must be increased approximately 30% in order to produce lesions of the same volume. Glial structures and the vascular system are more resistant to the action of ultrasound and, for threshold lesions, there is no interruption of the blood supply.

At acoustic intensities above about 2000 W/cm² and time durations less than 40 msec, the threshold lesion is believed to be produced by a cavitation mechanism. Such lesions were produced at 1 and 3 MHz and also by Pond[8] in the low-megahertz-frequency region. The cavitation region for threshold lesions shows (Fig. 1) an increase in cavitation threshold with increasing frequency, a phenomenon expected from all theories and mechanisms of acoustically induced cavitation in liquid media.[16] The character of the cavitation lesions, as discussed below, is considerably different from that due to the noncavitation mechanical and thermal mechanisms at doses near threshold, and the following differences are observed:

(1) For the cavitation mechanism, the lesion results from gross tissue damage, as opposed to the focal lesions which require approximately 10 min following ultrasonic exposure before histological evidence of the lesion emerges (as can be detected by a variety of staining methods and maximum magnification of the light microscope).

(2) Cavitation lesions may not be found at the focus of the transducer, as might be expected on the basis of volume distribution of energy. Instead, the lesions occur at interfaces between neural tissue and fluid-filled spaces, such as ventricles and blood vessels.

(3) Cavitation lesions do not exhibit the tissue selectivity characteristic of the focal lesions. It is generally believed[14] that the presence of cavitation nuclei is essential to produce weak spots in the body of the material exhibiting cavitation. Such nuclei may be very small gas bubbles, which can expand under appropriate stresses to resonant size before sudden collapse occurs. If such an hypothesis is adopted for the results presented here, presumably then there are few if any such nuclei in the body of neural tissue and they are more probably found in the fluid-filled regions such as blood vessels or in the ventricular system. In addition, since the ultrasound in this study was directed to avoid major blood vessels and the ventricles, and the cavitation apparently did not necessarily occur at the focus of the ultrasonic transducer, the intensity at the cavitation sites would be significantly lower than expected at the focal region of the transducer. As there is no information available on the intensity at the cavitation sites, the data plotted in Fig. 1 are those calculated to have been present in the focal region in the absence of cavitation.

Lesions produced with doses of ultrasound sufficient to develop cavitation in the cat brain are shown in Figs. 2, 3, 4, and 5. The adjacent 10-μ-thick brain sections shown are stained with Weil (on the right for white matter) and cresylecht violet (on the left for gray matter). All of these lesions were produced with 1-MHz ultrasound at a peak intensity of 5000 W/cm²

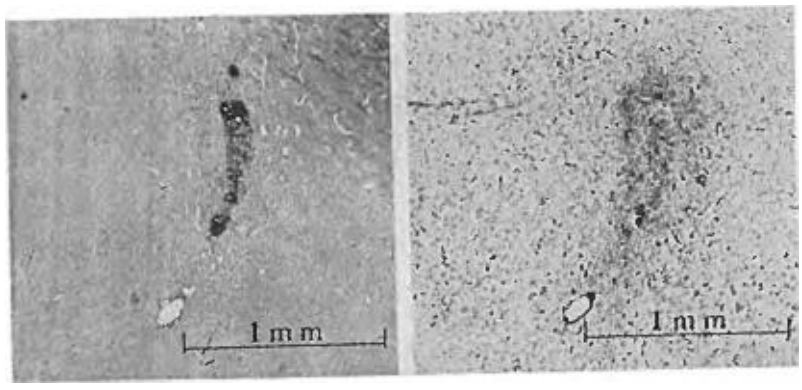

FIG. 2. Ultrasonically induced cavitation lesion in the post subicularis region of the cat brain.

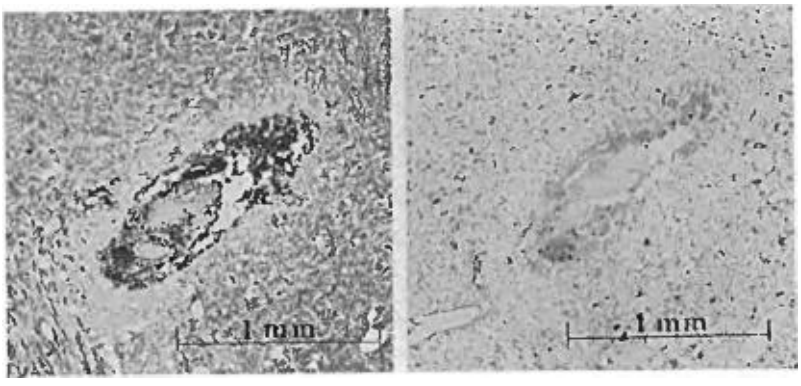

FIG. 3. Ultrasonically induced cavitation lesion in the mesencephalic reticular formation of the cat brain.

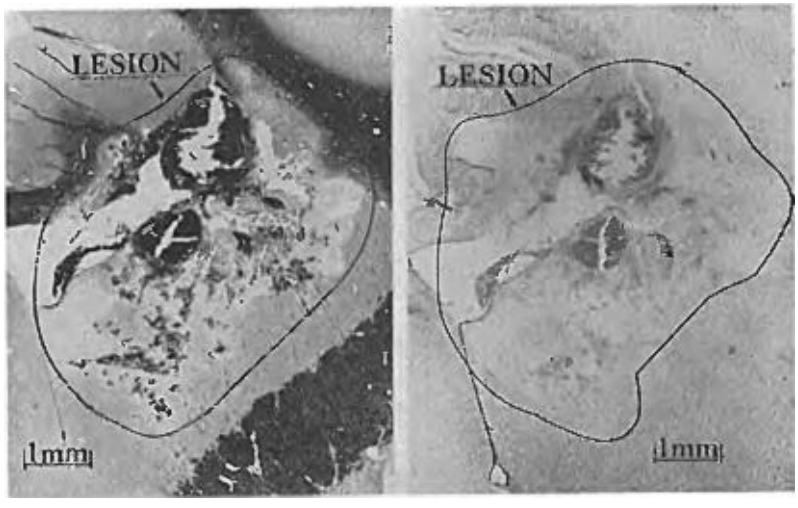

FIG. 4. Ultrasonically induced cavitation lesion at the interfaces of the ventricle, the corpus callosum, and the caudate nucleus of the cat brain.

at time durations of exposure between 25 and 200 msec. The different appearance of the lesions, in terms of a qualitative severity differentiation, is due to different times of exposure of the tissue to the focused ultrasonic beam. As the cavitation mechanism requires nuclei for initiation, a direct correlation between lesion severity and exposure time (for a fixed sound intensity), particularly near threshold, is not necessarily expected. Cavitation-mediated lesions developed at other suprathreshold intensities and exposure times exhibit the same general characteristics.

Figure 2 shows a lesion of 1-mm length in the post subicularis region of the left brain of the cat. Both stains show large erythrocyte concentrations throughout the entire central region of the lesion in which no intact blood vessels appear. Exterior to the central region is an area in which erythrocytes are not present and the blood vessels are intact, though the background matrix is in disarray compared to the adjacent normal tissue. Any identifiable neurons remaining in the lesion region show various degrees of abnormality, one predominant feature being lack of evidence of cytoplasm. The lesion

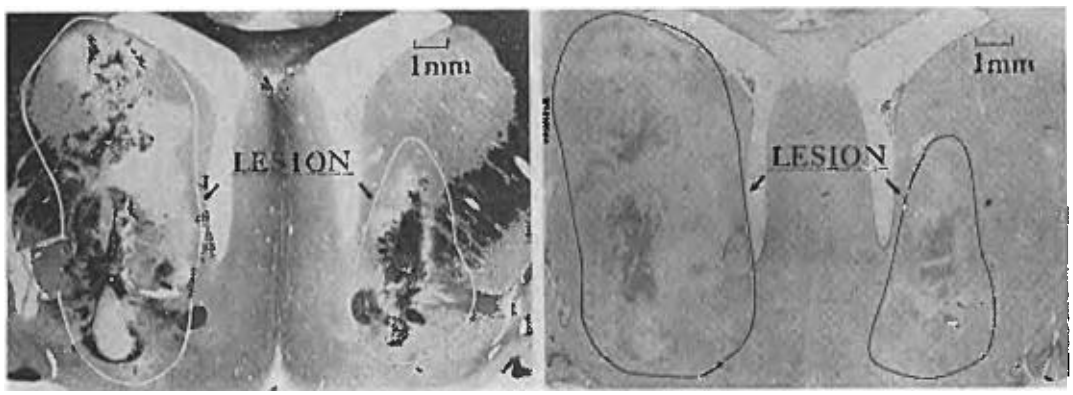

FIG. 5. Ultrasonically induced cavitation lesion in the internal capsule and the caudate nucleus of the cat brain.

exhibits a curved shape, indicating a preferential orientation, due perhaps to small blood vessel placement. This lesion does not show the great disarray of material and the disrupted boundary between normal and altered tissue seen in heavier lesions. In view of the geometrical position of this lesion in the brain, the region of most severe damage appears to be several millimeters posterior to the region of the maximum beam intensity, while in the vertical and lateral directions the lesion appears at the intended maximum beam intensity position.

The lesion shown in Fig. 3 is just lateral to the central griesum in the mesencephalic reticular formation. It is 2 mm long and its axis is at an angle of approximately 45° with respect to the vertical axis of the brain. A preferential position with respect to the blood vessel orientation is apparent in this case. The lesion has a large central region well populated with erythrocytes, large irregularly shaped holes and tissue spaces in which the background matrix structure is missing. There is a narrow region surrounding the central area in which the matrix is in disarray—an increased number of vacuoles appear—and some small satellite areas appear around blood vessels showing erythrocyte dispersement into the surrounding tissue. These satellite regions are interpreted as possible cavitation sites at the blood-vessel interface. No intact neurons appear in any part of the lesion. Much glial and other cellular debris is seen in the narrow surrounding region, although a few normal-appearing glia are present. The lesion is at the maximum beam intensity position vertically, but is more medial by 3 mm and more posterior by about 2 mm than the maximum intensity region.

A much heavier lesion is shown in Fig. 4. This lesion has no definable boundary and is approximately 6×6 mm in size in the vertical and lateral directions. Regions involved in the lesion are interfaces with the left lateral ventricle, the left corpus callosum, and a considerable portion of the left caudate nucleus. There are many large hemorragic and torn regions intermingled with tissue areas which show the matrix in complete disarray and a large number of vacuoles present. Evidence of blood vessel disruption in the peripheral lesion region is quite apparent. All neurons are missing from the region in the caudate nucleus except for one interspersed area in which the tissue appears normal. This area is irregular in outline and represents no more than 1% of the entire lesion.

Figure 5 shows two large lesions in the internal capsule with invasion of the caudate nucleus of both sides of the cat brain. Both lesions are centered in the region of highest intensity of the sound beam. Lesion boundaries are nonuniform in shape and large areas of erythrocyte concentration are characteristic. Large clefts and holes also appear and there are no normal cellular elements apparent in the lesion area. Cellular debris is in evidence in the lesion area and blood vessels in the more central regions are disrupted. Many blood vessels in satellite areas show slight (less than 50 erythrocytes) to large numbers of erythrocytes dispersed into the surrounding tissue.

For comparison purposes, the cat-brain section of Fig. 6 is presented to show selective white-matter lesions (noncavitation). The mammillothalamic tract on the right side of the Weil-stained section is completely missing and the fornix tract, on the same side, has been partially interrupted. A detailed study of the tissue section shows that the lesion is restricted to the fiber tracts indicated. This animal was sacrificed much longer after irradiation than those of Figs. 2 5.

The results of the present study, together with the data of Pond[4] and Lele,[3] comprise the most comprehensive set of information available on the histologically observed reaction of mammalian central nervous tissue to intense ultrasound *in vivo*. Thus, it appears profitable to consider these data in terms of the hazard ultrasound presents when employed as a medical diagnostic tool. Hill[15] has determined the output of several ultrasonic instruments manufactured for use as diagnostic tools in medicine and finds that the upper limit of the acoustic output is of the order of 10^2 W/cm^2,

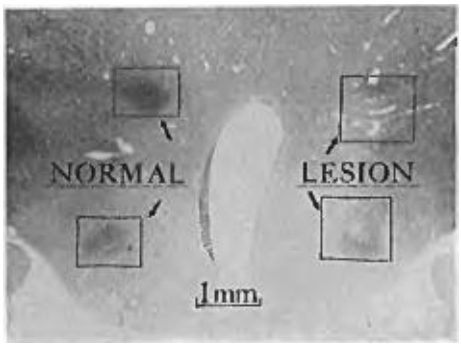

FIG. 6. Ultrasonically induced selective white-matter lesion (noncavitation) in the mammillothalamic tract and the fornix tract of the cat brain.

with pulse lengths of 10^{-6} sec and pulse repetition rates of 10^3 pps. Fry *et al.*[16] showed that repeated pulses of ultrasound of low duty cycle and short pulse duration do not produce permanent functional changes even after prolonged exposure. Thus, the usual pulse amplitudes and durations employed in diagnostic work appear to be several orders of magnitude below the doses required to produce threshold histologically observed lesions in the mammalian central nervous system and this tissue appears to be among the most sensitive of adult tissues.[9] From these considerations, it would appear that there is little likelihood that ultrasound provides a hazard when employed for medical diagnostic purposes with the regime described above.

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[1] W. J. Fry, "Intense Ultrasound in Investigations of the Central Nervous System," in *Advances in Biological and Medical Physics*, J. H. Lawrence and C. A. Tobias, Eds. (Academic, New York, 1958), Vol. 6, pp. 281–348.

[2] W. J. Fry and F. J. Fry, "Fundamental Neurological Research and Human Neurosurgery Using Intense Ultrasound," IRE Trans. Med. Elec. ME7, 166 181 (1960).

[3] L. Basauri and P. P. Lele, "A Simple Method for Production of Trackless Focal Lesions with Focused Ultrasound," J. Physiol. 160, 513 534 (1962).

[4] J. S. Manlapaz, K. E. Astrom, H. T. Ballantine, Jr., and P. P. Lele, "Effects of Ultrasonic Radiation in Experimental Focal Epilepsy in the Cat," Exp. Neurol. 9, 502–511 (1964).

[5] M. Oka, T. Okumura, H. Yokoi, T. Murao, Y. Miyashita, K. Oka, S. Yoshitatsu, K. Yoshioka, H. Hirano, and Y. Kawashima, "Surgical Application of High Intensity Focused Ultrasound," Med. J. Osaka Univ. 10, 427–442 (1960).

[6] F. Dunn, "Physical Mechanisms of the Action of Intense Ultrasound on Tissue," Amer. J. Phys. Med. 37, 152–156 (1958).

[7] J. W. Barnard, W. J. Fry, F. J. Fry, and R. F. Krumins, "Effects of High Intensity Ultrasound on the Central Nervous System of the Cat," J. Comp. Neurol. 103, 459–484 (1955).

[8] J. Pond, "A Study of the Biological Action of Focused Mechanical Waves (Focused Ultrasound)," PhD thesis, Univ. of London (1968).

[9] T. F. Hueter and W. J. Fry, "Ultrasonics: Central Nervous System Changes Produced by Focused Ultrasound," in *Medical Physics*, O. Glasser, Ed. (Year Book, Chicago, 1960), Vol. III, pp. 671–678.

[10] F. Dunn, P. D. Edmonds, and W. J. Fry, "Absorption and Dispersion of Ultrasound in Biological Media," in *Biological Engineering*, H. P. Schwan, Ed. (McGraw-Hill, New York, 1969), Chap. 3, pp. 205–332.

[11] R. M. Macleod and F. Dunn, "Effects of Intense Noncavitating Ultrasound on Selected Enzymes," J. Acoust. Soc. Amer. 44, 932–940 (1968).

[12] A. Coble and F. Dunn (to be published).

[13] H. H. Jasper and C. Ajmone-Marson, "A Stereotaxic Atlas of the Diencephalon of the Cat," Nat. Res. Council of Canada (1960).

[14] H. G. Flynn, "Physics of Acoustic Cavitation in Liquids," in *Physical Acoustics*, W. P. Mason, Ed. (Academic, New York, 1964), Vol. I, Pt. B, Chap. 9, pp. 57–172.

[15] C. R. Hill, "Acoustic Intensity on Ultrasonic Diagnostic Devices," Proc. First World Cong. on Ultrasonic Diagnostics in Med. (to be published) (1970).

[16] W. J. Fry, V. J. Wulff, D. Tucker, and F. J. Fry, "Physical Factors Involved in Ultrasonically Induced Changes in Living Systems," J. Acoust. Soc. Amer. 22, 867–876 (1950).