FOCAL DESTRUCTION OF NERVOUS TISSUE BY FOCUSED ULTRASOUND: BIOPHYSICAL FACTORS INFLUENCING ITS APPLICATION*

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(Plates 28 to 30)

(Received for publication, May 3, 1956)

It has recently been established (1–3) that focused ultrasound can produce discrete areas of tissue damage deep within brains of cats and monkeys. Such lesions have a great potential value for the neurophysiologist, and ultrasonic techniques may well have a place in the treatment of diseases of the human nervous system. In order to realize this potential, however, certain rigid criteria must be met. First, damage must be limited to the region selected for destruction. Second, this region must be rendered completely non-functional in a large percentage of cases. Third, the extent of the zone of destruction following any given irradiation dose must be predictable. The investigations to be reported here were designed to identify some of the biophysical factors which must be controlled if these criteria are to be satisfied.

Part I. Evaluation of Dosage Parameters

Principles of Ultrasonic Focusing.

The technique used in these experiments is illustrated in Text-fig. 1 which shows a schlieren photograph of a focused ultrasonic beam. A high frequency radio current activates a piezoelectric crystal. In front of this crystal is a polystyrene lens through which the ultrasonic waves pass and are concentrated into a focal region of high intensity. It is important to note that this is a focal region rather than a focal point. The focal region is conveniently defined as that volume within which the intensity is at least one-half of the "peak" intensity calculated to be present at an assumed focal point. In principle, then, one desires to utilize an intensity

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1 In ultrasonics, intensity is defined in terms of the energy (in joules) flowing in 1 second through unit area; intensities ranging from 10 to 3 thousand watts/cm² are being employed in our work.
and irradiation time which are sufficient to destroy nervous tissue within the focal region but at no other point within the path of the beam.

This converging beam of ultrasound can be compared to the focusing of a light beam and the same optical principles apply; i.e., the diameter of the focal region depends on the aperture of the focusing device and cannot be smaller than the wavelength (4). Focusing may be achieved in several ways: using parabolic reflectors, lenses or multiple beams aiming at one point. All are being used, but a single lens of large aperture seems to have the advantages of compactness, ease of adjustment, and optimal intensity gain.

The dimensions of the focal region as delineated by the so called “half-power points” will vary with the frequency: the higher the frequency, the smaller the focal region. According to our measurements at 1 megacycle per second the dimensions of the focal region are about 2.5 mm in width and 20 mm in length. At 2.5 megacycles per second, this region measures 1 mm in width and 8 mm in length. It is important to point out, however, that the actual size of the lesion produced at the focus depends not only on the geometrical distribution of the intensity, as specified above, but also on the magnitude of the intensity to which the tissue is subjected. This is demonstrated in Text-fig. 2 which shows the intensity distributions along the axis of the focused beam for three different power outputs from the crystal. The axial half-power points are at ± 4 mm, a critical intensity level above which tissue damage occurs is indicated. As we shall see later, this threshold level, in turn, depends on the duration of the irradiation and on the temperatures prevailing at the site of irradiation: lower thresholds are associated with longer irradiation times or with higher temperature levels. We note that for curve (C) the peak intensity stays below the damage threshold and thus no lesion is obtained. The higher intensities of curve (B) will produce lesions of 4.5 mm, axial extension. If the power output is further increased, curve (A) may be obtained, for which the axial extension of the lesion becomes 7.5 mm. Similar considerations apply to the lateral extension of the focal lesion.

A lesion can always be enlarged, but obviously can never be reduced in size. To obtain the smallest possible lesions, the majority of our animal work has been done at 2.5 mc., although other investigators in this field have used a frequency in the neighborhood of 1 mc. At the
higher frequency, however, ultrasound is more strongly attenuated as it passes through tissue, and for work deep within the human brain it might be difficult to develop sufficient focal in-

\begin{center}
\begin{tabular}{|c|c|c|}
\hline
INTENSITY LIMITS OF & LESION & \\
DAMAGE & DIAMETER & \\
\hline
A HIGH & 0 & 7.5 mm. \\
B MEDIUM & X & 4.5 mm. \\
C LOW & 0 & 3.0 mm. \\
\hline
\end{tabular}
\end{center}

Text-Fig. 2. Axial intensity distribution of focused beam for three output levels. Damage occurs wherever the intensity exceeds the critical level, indicated by shading.

tensity at 2.5 mc. to destroy tissue. Actually, it is possible to specify an optimal frequency for each tissue depth (3).

Observations after Ultrasonic Irradiation of the Spinal Cords of Mice.—

Text-fig. 3 shows our apparatus in position for the irradiation of the spinal cords of mice in the studies of dosage parameters discussed below. The cabinet at the left houses the electrical generating equipment and control circuits. Radio frequency power up to about 50
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watts is passed through a cable to the transducer, depicted in Text-fig. 4, which consists of a quartz crystal, a polystyrene lens, and a water-filled applicator, which is positioned over the mouse. The end of the applicator cone is sealed by a thin sound transparent membrane. The center of the focal region is located outside of the applicator at a distance of 3 mm. from the membrane. This distance corresponds to the average distance of the center of the cord from the surface of the skin.

Questions under study with this apparatus included: (a) the ultrasonic dose (intensity and duration of ultrasonic exposure) required to produce a limited region of damage in the mouse spinal cord, (b) the correlation between ultrasonic dose and the extent of damage, (c) the interdependence of ultrasonic intensity and irradiation time as measured by a physiological end-point (paraplegia), (d) whether heating of tissue, due to absorption of ultrasonic energy at the focal region, was the predominant cause of tissue damage.

Membranes made of polystyrene or cellophane were found unsuitable because poor wetting led to cavitation breakdown at the membrane-liquid interfaces if high sound intensities were used. A special animal membrane ("goldbeaters' skin") procured from the Central Scientific Company, Chicago, Illinois, has proved highly successful in our work.
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Of the many different parameters that can be varied when exposing tissue to ultrasound, the intensity and its distribution in time have proven to be the most important. In this respect the reaction of tissue to ultrasound is similar to that seen after exposure to other potentially noxious agents (e.g. heat, x-radiation, or drugs).

With pulsed ultrasound, the temperature rise due to absorption of ultrasonic energy at the focus can be kept within certain limits (5) by providing sufficient time between the pulses for the dissipation of heat. Pulsing or similar forms of intermittent irradiation thus expose the tissue to very high mechanical strains without reaching excessive temperatures. To demonstrate this possibility a number of controlled experiments have been devised (6). Whereas mere heating would coagulate all types of tissues indiscriminately, mechanical wear and tear may affect tissues selectively (7).

It is convenient to study dosage questions with small animals, such as frogs or mice, that are readily available in the large numbers required to obtain statistically meaningful results. We have irradiated the spinal cords of mice, with paralysis of the hind legs as a physiological end-point. These pilot experiments were performed as follows:

Adult mice (weight about 30 gm.) were anesthetized with ethyl chloride and the skin over the middorsal region was shaved. The ultrasonic irradiator was applied over 9th and 10th vertebral bodies. The timing of the pulses was as follows: pulse period (pp) ~ 1.0 second; pulse width (pw) ~ 0.4 second; pulse number (N) ~ 1 to 100. Two frequencies, 1 mc and 2.5 mc, were used with ultrasonic intensities ranging from 40 to 2000 watts per cm.². Paraplegia was taken as an indication of complete cord destruction while paralysis of one lower extremity indicated localized damage.

Some typical results of our initial experiments using 30 pulses are listed in Table I. It was possible to choose optimal parameters of irradiation to produce paraplegia without gross evidence of skin damage and with no immediate effect on mortality.

Both the skin damage and the high mortality observed at 1 mcps were undoubtedly due to extension of the critical dosage level along the axis of the beam as the intensity was increased (see Text-fig. 2). This widening of the zone of damage is more pronounced at 1 mcps because of the poorer focusing at this frequency compared with 2.5 mcps, using the same lens in both cases.

The mice rendered paraplegic under optimal dosage conditions ate and drank well, were active within their physical limitations, and in general did not appear ill. Paraplegia was thought to be complete when the hind legs were not used for walking, showed no reaction to a painful stimulus applied below the level of irradiation, and showed no reaction to a painful stimulus applied cephalad to the point of irradiation and with sufficient intensity to elicit movements.

³ The effect of pulsing on the amount of heating produced at the focus of the 2.5 mc beam is illustrated by the following data: a temperature rise of 2.2°C. per 100 watts/cm.², as obtained after 30 seconds of pulsed irradiation at pp = 1.0 second and pw = 0.4 second, requires only 5 seconds of continuous ultrasound.
of the fore limbs. The urinary bladders of the paraplegic mice were always distended and there was continual dribbling of urine. This situation resembled the overflow incontinence seen in paraplegic humans.

In the mice used for these experiments the spinal cord lies about 3 mm. below the skin. The production of paralysis without skin damage gave evidence of satisfactory control of critical intensity along the axis of the ultrasonic beam. Information as to lateral control was provided when it was found that monoplegia resulted from irradiation of the mouse spinal cord 1 mm. to either side of the midline.

Histologic examination of mouse spinal cords exposed to ultrasound presented a variable picture. Three representative sections are reproduced in Figs. 1 to 3. Although some of the variations in our histological findings may have been due to slight inconsistencies in the centering of the ultrasonic beam (as, for instance, the case illustrated by Fig. 2, in which one quadrant of the spinal cord escaped injury), certain other phenomena must be explained otherwise. Fig. 3, for example, shows evidence suggestive of differences in susceptibility to ultrasonic injury between the heavily myelinated fiber systems of the white matter on the one hand, and the predominantly non-myelinated grey matter on the other hand. Furthermore, findings illustrated by Fig. 1 suggest that ultrasound dosages high enough to destroy the neural structures of the spinal cord may leave the meningeal coverings and the segmental nerve roots intact. Likewise, it is not uncommon to find numerous apparently intact glia nuclei in areas where neural structures have been completely disintegrated.

Table I

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Range of intensities</th>
<th>Incidence of paralysis</th>
<th>Skin reaction</th>
<th>General reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mcps</td>
<td>60-200 w/cm²</td>
<td>30-80 per cent</td>
<td>Wheals in most animals, burns at the higher intensities</td>
<td>Many animals sick following irradiation. At highest dosage 10 out of 15 dead within 5 days</td>
</tr>
<tr>
<td>2.5 mcps</td>
<td>400-1000</td>
<td>30-80</td>
<td>Occasional wheal at highest intensity</td>
<td>No apparent illness, low mortality</td>
</tr>
</tbody>
</table>

Statistical Evaluation of Intensity-Time Relationship.—After the preliminary studies described above, the probability of ultrasonic damage to the mouse spinal cord was determined for different “pulse numbers” (i.e. exposure times) as a function of the mechanical strain amplitude. All these experiments were performed at a frequency of 2.5 mcps.
Some typical data are shown in Text-fig. 5, in which each point represents a sample of about 30 mice. The probability of paralysis (in per cent) is plotted versus crystal driving voltage, which in turn is proportional to the mechanical strain amplitude at the ultrasonic focus. The slope of the resulting sigmoid curves as well as the probable error of each sample indicates a fairly large biological variation of susceptibility to the ultrasound. The best defined point is the median of the distribution curve, as obtained from a reduction of all the data to a best fitting line on probability paper, assuming a normal distribution. From a determination of the number of pulses required at different voltage settings to paralyze 50 per cent of the animals in any given group of mice one may find a general relationship between sound intensity and the associated necessary exposure time.

For a graphic presentation of this dosage relationship, it is convenient to plot the reciprocal of the pulse number versus a quantity proportional to the square root of the sound intensity, such as the crystal driving voltage. The result is presented in Text-fig. 6 which also shows the final local temperature rise above body temperature associated with each pair of irradiation parame-
Text-Fig. 6. Curve representing the combination of pulse number and sound intensity required to produce paralysis of the hind legs in 50 per cent of mice subjected to irradiation at the parameters charted. Also indicated are the final temperature rises resulting from any such combination. V₀ equals 280 RF volts (see text).
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ters. We shall see that the temperature level achieved at the end of the ultrasonic irradiation has a considerable influence on the duration of ultrasound necessary to produce a given effect at a given intensity level.

The graph of Text-fig. 6 suggests that under the conditions prevailing in this experiment (mice at normal body temperature, pulsed irradiation, frequency 2.5 mcpp) there are two different regions with regard to the manner in which the tissue responds to the ultrasonic stresses:

At sound intensities below 400 w./cm.\(^2\) the dosage is approximately proportional to the product of pulse number and the excess of voltage above a threshold value \(V_\theta\). Below \(V_\theta\) no paralytic effect was observed even after prolonged exposure to ultrasonic pulses. Above \(V_\theta\) the dosage appears to follow a simple amplitude \(\times\) time law, as indicated by the dashed straight line intersecting the abscissa at \(V_\theta\). In this region the local heating due to the absorption of ultrasound in the focal region seems to have little effect on the required dosage: the dosage curve cuts across the temperature curves in Text-fig. 6. At sound intensities above 450 w./cm.\(^2\) the irradiation times required for 50 per cent paralysis become increasingly shorter than may be expected from an amplitude \(\times\) time law. At the same time the dosage curve approaches asymptotically one of the temperature curves, in this experiment the one for a rise of about 30°C. above normal body temperature.

Whereas the response to lower intensities supports the notion of a mechanical destruction of the tissue by ultrasound, the trend apparent with higher intensities suggests an increasing effect of tissue heating. At intensities above 1500 w./cm.\(^2\) the heating appears to be sufficient to cause coagulation. We are thus led to conclude that the mechanical effect is increasingly overshadowed by the heat effect when the sound intensity at the focus becomes very high. A similar course of events was observed when producing discrete circumscribed lesions in cat brains. All of our experiences to date indicate that close attention must be paid to the temperature rise produced by focused ultrasound; this depends on such biological factors as the absorption coefficient of the tissue irradiated, the blood flow, and the ambient tissue temperature.

It is interesting to note that some structures in the mouse cord, such as the ependyma and pia-arachnoid appeared intact, even when the adjacent cord substance had been almost completely destroyed (see, for instance, Fig. 1). This degree of selectivity would be difficult to reconcile with an effect based solely on thermal denaturation.

Part II. Stereotaxically Placed Ultrasonic Lesions in the Diencephalon of the Cat

The knowledge gained by the dosage studies in mouse cords, reported in Part I of this paper, has provided a suitable basis for an attack on tissue structures of greater anatomical complexity in the brains of larger mammalian animals such as cats or monkeys and as a final goal, the human brain. Of these, the cat

The temperature curves of Text-fig. 6 were determined experimentally by inserting small thermocouples into the mouse cord at the site of focal irradiation.
The brain has been mapped most thoroughly (8) and has therefore been adopted as a convenient target material for a determination of the accuracy and reproducibility with which stereotaxically placed lesions can be produced at a desired site by focused ultrasound.

Text-Fig. 7. Schematic diagram of stereotaxic irradiation procedure, with cat fixed in Horsley-Clarke machine and ultrasonic focus located at coordinates frontal 7, lateral 10, vertical +3 (lateral geniculate body.)

The use of stereotaxic procedures implies shooting blindly at a target within the brain, guided only by the coordinates of an atlas based on measurements from external reference points on the skull of the animal (Text-fig. 7). However, the ultrasonic method requires removal of a portion of bone from the skull as a port of entry of the converging beam to eliminate the distorting and attenuating effects of the bone (9, 10). The dura mater need not be opened and, moreover, we have found it possible to apply the ultrasound through the healed scalp after completion of the bone surgery. Such a modification of the procedure seems to have certain advantages for the use of focused ultrasound in human neurosurgery.
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Technique of Stereotaxic Irradiation.—

In designing a suitable apparatus for aiming the ultrasonic beam we have been guided by the following requirements: flexibility, mobility, compactness, and low cost. The result is a unit combining a wooden cat support, the counter-balanced column of a mobile x-ray unit, a calibrated cross-feed of the type commonly used with drill presses, and a commercial Horsley-Clarke machine. Text-figs. 8 and 9 illustrate this apparatus, which has proved satisfactory in irradiations of more than 80 cats.

The cross-feed provides accurate lateral and vertical positioning of the transducer; the frontal plane of the irradiation is adjusted by moving the cat table itself. For zeroing, a pointer may be attached to the lens of the transducer, and the 3 coordinate movements may be adjusted so that the pointer tip coincides with the center of the ear bar line of the Horsley-Clarke device. For the skull surgery, the cat table may be lowered to a convenient position, by means of the counter-balanced x-ray column, without changing the final coordinate settings.

Since the passage of ultrasound is effectively blocked by even the thinnest layer of air, a suitable coupling medium must be provided in the space between the lens and the brain. This may be achieved either by an applicator cone of the kind described in Part I, filled with degassed saline, and sealed with a thin membrane, or by an open pan whose lower rim is temporarily attached to the scalp of the animal, the pan subsequently being filled with degassed saline into which the lens is immersed. We have used the open pan arrangement for the target studies to be reported below, but have also determined the usefulness of the sealed applicator technique whenever we have irradiated through the scalp, after previous removal of the necessary amount of skull bone.

The advantage of pulsed irradiation in reducing the time rate of the temperature rise in the focal region has been pointed out in Part I of this paper. The relationship between pulse numbers and focal intensity required to produce small lesions in the white matter of cat brains is quite similar to the one found from the “50 per cent paralysis” criterion in the irradiations of mouse cords. This is illustrated in Table II which shows the intensities required to obtain equivalent lesions, for a variety of pulse numbers in cat brains as well as in mouse cords. The agreement is surprisingly good, considering the differences of anatomical location and irradiation techniques in both cases.

Evaluation of Dosage Correction Factors.—In Part I of this paper, under Principles of Ultrasonic Focusing, we have given values for the dimensions of the focal region, as produced by our focusing applicators. From the cross-sectional area (in square centimeters) of the beam constriction at the focus, and from the ultrasonic power (in watts) transmitted through this area, a number may be derived for the maximum intensity (in watts/cm.²) at the center of the focal region. The focal geometry, the beam power and the resulting peak focal intensity are most conveniently measured in a standard low loss.

5 Manufactured by Labtronics, Inc., Chicago.
6 A sufficient amount of degassing is achieved by autoclaving the saline in pyrex bottles, fitted with Fenwal pour-o-vac seals.
7 Here and in the following discussion “small” designates a lesion, usually of ellipsoidal shape, characterized by a product of smallest × largest dimension of less than 6 mm.², “medium” designates a lesion for which this product has a value between 6 and 12 mm.², and “large” applies to lesions greater than 12 mm.².
TEXT-FIG. 8. View of stereotaxic irradiation system: at left the counter-balanced vertical column. Top center the cross-feed supporting the transducer head. Pointer attached to transducer face indicates focal point, which is zeroed at center of ear bars of Horsley-Clarke device shown below.
medium such as degassed water. Greatest accuracy of this beam calibration is essential for reproducible lesion making.

However, once the applicator is calibrated in water, additional corrections

\(^8\) Methods for beam calibration have been discussed (3).
must be applied to allow for the acoustic properties of the tissues intervening between the applicator face and the site of the desired lesion, and the thermal and structural properties of the tissues at the lesion site.

Changes in Beam Geometry.—One might expect some refraction of the sound waves as they pass from coupling liquid (saline) through the dura into the brain. It would appear, however, that this effect is negligible, since the critical acoustic constants, namely the specific density and sound velocity, in brain are about the same as in water. The necessity of removing a portion of the overlying skull, which would otherwise produce drastic changes in beam geometry, has been pointed out before.

Changes in Beam Strength.—Whereas water or saline transmit the ultrasound without appreciable absorption, brain tissues subtract energy from a transmitted ultrasonic beam. These losses are the cause of the tissue heating mentioned above. In brain tissues the intensity decreases exponentially by amounts of about 10 per cent for each 2 mm. of brain traversed at 2.5 mcps, and for each 5 mm. of brain traversed at 1 mcps (11). It is therefore mandatory to determine the mean depth of the lesion site for each irradiation, and to compensate for the loss in beam strength by an appropriate increase of the power emitted by the applicator.

### Table II

Comparison of Equivalent Dosages in Cat Brains and Mouse Cords (2.5 mcps)

<table>
<thead>
<tr>
<th>No. of pulses applied</th>
<th>Cat No.</th>
<th>Lesion size</th>
<th>Peak Intensity at Lesion Center</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brain, white matter (small lesion)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>w/cm.²</td>
</tr>
<tr>
<td>10</td>
<td>5-0 B</td>
<td>1 × 2</td>
<td>870</td>
</tr>
<tr>
<td>20</td>
<td>5-0 C</td>
<td>1 × 2</td>
<td>520</td>
</tr>
<tr>
<td>30</td>
<td>6-5 B</td>
<td>1 × 1.5</td>
<td>820</td>
</tr>
<tr>
<td>30</td>
<td>6-0 A</td>
<td>1 × 1</td>
<td>580</td>
</tr>
<tr>
<td>30</td>
<td>5-4 A</td>
<td>1 × 5</td>
<td>600</td>
</tr>
<tr>
<td>30</td>
<td>5-8 A</td>
<td>1.5 × 2.5</td>
<td>600</td>
</tr>
<tr>
<td>30</td>
<td>5-8 B</td>
<td>1.5 × 2.5</td>
<td>600</td>
</tr>
<tr>
<td>35</td>
<td>6-9 A</td>
<td>1 × 5</td>
<td>510</td>
</tr>
<tr>
<td>40</td>
<td>6-9 B</td>
<td>1.2 × 1.5</td>
<td>400</td>
</tr>
<tr>
<td>45</td>
<td>7-3 B</td>
<td>1 × 1</td>
<td>400</td>
</tr>
<tr>
<td>45</td>
<td>7-4 D</td>
<td>1 × 1.5</td>
<td>400</td>
</tr>
<tr>
<td>50</td>
<td>7-8 A</td>
<td>1.5 × 4</td>
<td>390</td>
</tr>
<tr>
<td>60</td>
<td>8-2 B</td>
<td>1.5 × 2.5</td>
<td>330</td>
</tr>
</tbody>
</table>
Temperature Coefficient of Damage Threshold.—The influence of the ultrasonically produced temperature rise on the development of tissue damage has been pointed out above, in connection with the graph of Text-fig. 6. Obviously, the final temperature level attained at the lesion site and at the end of the ultrasonic exposure depends on a number of physiological factors. They include the temperature of the brain at the start of the irradiation, the vascularity of the target area, and the degree to which heat is carried away by the blood flow. To explore the relative role of these factors we have performed a number of experiments in which the temperature of the brain was temporarily lowered to 31°C. or raised to 40°C. As expected, the same ultrasonic dosage produced a smaller lesion at the lower temperature and vice versa.

In another experiment both carotid arteries were occluded during pulsed irradiation of about 50 seconds total duration. Small thermocouples inserted stereotaxically into the lesion site revealed higher temperature rises in the occluded case than in the control. The higher temperatures were associated with larger lesions.

It therefore appears necessary to carry out irradiations at normal conditions of temperature and blood flow, whenever the greatest accuracy is desired, as in neuro-anatomical studies.

Structure-Dependent Tissue Susceptibility.—It has been demonstrated that certain tissue components (e.g. cortical grey matter and the thalamic nuclei) are more resistant to a given dose of ultrasound than others (e.g. white matter). The cause of these differences in susceptibility are still unknown, although they seem to be associated with regional tissue structure rather than with specific properties of the individual components such as nerve cells and fiber tracts.

It follows from these findings that the dosage requirements depend on the type of tissue to be destroyed, and that there may be neuro-anatomical limitations upon the placement or shape of a lesion. For example, it is generally easier to selectively damage a region of white matter surrounded by grey matter, such as in the mammillo-thalamic tract or the internal capsule, than to damage an aggregate of nerve cells imbedded in white matter, such as the lateral geniculate body.

Measurement of Tissue Damage.—Typical examples of medium to large lesions, as they appear if the animal is sacrificed 7 to 10 days post irradiation, appear in Fig. 4. This cat was exposed to pulsed ultrasound of 2.5 megacycle frequency at two different focal intensities: 710 watts/cm² on the right and 950 watts/cm² on the left. The histological section from the left was stained for myelin and that from the right with thionine for demonstration of any cell damage. Lesions as large and as old as those in Fig. 4 can be easily identified by macroscopic inspection of the sectioned brain. Histological examination after the 1st week reveals only the non-specific changes that follow any gross trauma to the brain.

To delineate the specific effects of ultrasonic irradiation some of the histologic studies must be undertaken soon after treatment. Fig. 5 shows the appearance of a lesion 24 hours post irradiation. The area chosen as the target was located at stereotaxic coordinates frontal 10, lateral 8, vertical 2 (Cat 3–6 myelin stain).

See reference 8.
In this case the periphery of the lesion revealed a large vertical zone of diffuse pathology, involving one lateral part of the subthalamus, extending through the internal capsule ventrally into the medial part of globus pallidus and optic tract. In this zone mild gliosis prevails, with scattering of poly- and mononuclear cells. There are ghost cells among the nerve cells in this region. Immediately caudal to this level a large well defined spindle-shaped area appears, occupying mainly the internal capsule and optic tract, within which nerve fibers have been destroyed and nerve cell bodies are completely missing. At its largest extent the lesion measures approximately $7 \times 3$ mm. There is no actual tissue defect in this region. Surrounding the lesion is a narrow zone of gliosis.

It has been determined by standard histological techniques that the earliest evidence of a lesion may usually be seen about 10 minutes following irradiation and that about 1 to 2 hours later the final outline of the tissue reaction is more or less apparent (12). The further development of the lesion proceeds according to the normal patterns of necrosis and histolysis followed by scar formation. We have, therefore, sacrificed the animals at a standard time of 1½ hours following irradiation.

The large amounts of time and effort involved in histological preparations are a considerable handicap in any attempt to establish the optimal experimental conditions and to evaluate the variations in technique. We have, therefore, searched for other methods to outline the boundaries of lesions 1 hour old.

A promising solution to this problem resulted from a study of the effects of intense ultrasound on the blood-brain barrier (13). To ascertain to what extent the permeability of the capillary walls is altered in the focal region we injected radioactive phosphorus ($^{32}\text{P}$) intravenously at various intervals after ultrasonic irradiation. Autoradiographs of brain sections indicated a considerable breakdown of the blood brain barrier at the site of the lesion. This finding suggested the possibility of using a vital stain, such as trypan blue which might flow out of the damaged capillaries and thus mark the location and shape of an ultrasonic lesion. Histological examination of lesions stained by trypan blue showed this method to be a reliable index of the area of tissue damage.

Fig. 6 shows the result of an irradiation of the lateral geniculate body in a cat which received 20 cc. of trypan blue suspension (13) intravenously 1 hour following the exposure to ultrasound, and 5 minutes later was sacrificed and perfused with saline formaldehyde.

**Determination of the Accuracy of Placement of Focal Ultrasonic Lesions.**—Because the size of a lesion is influenced by small changes in the ultrasonic focal intensity (see Text-fig. 2) and by local variations of heat dissipation within the brain it follows that the amount of focal tissue damage should be subject to greater variability than the locus of the lesion. This expectation was borne out by experiments in which we exposed groups of cats to the same ultrasonic dosage. The consistency in size of very small lesions (lateral diameter about 1 mm., axial length about 2 mm.) was considerably less than that of medium or large lesions; the effect varied from no lesion at all to a lesion twice the desired dimension. The scattering of size seemed to be larger in white matter than in grey matter, and smaller in relatively superficial than in more deeply situated lesions.
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Other investigators\(^7\) have differentiated between light, medium, and heavy lesions by their morphological appearance rather than by their size. One should recognize the difficulty of defining such terms as "size" and "appearance" of a damaged tissue region undergoing rapid changes as a function of time. Moreover, different staining or tracing techniques may yield different outlines of an affected region during the early and intermediate stages of a lesion.

Variations in the final size of a lesion amounting to plus or minus a few millimeters in diameter, are of some concern to the neuro-anatomist who desires to study neural pathways, although they do not invalidate the ultrasonic method for such studies. As to possible applications of the ultrasonic method in human neurosurgery, we believe that the observed variability will prove to be unimportant in view of the larger size of structures in the human brain.

Results with the Lateral Geniculate Body.—Since accuracy in placement of a lesion is of prime importance for fruitful applications of this method, a statistical approach to the evaluation of accuracy was necessary. In the experiment to be reported here focal destruction within the lateral geniculate body of the cat was attempted. This structure was chosen as a target because it can be identified in the gross as a grey mass within white matter, it lies deep within the brain and is of fairly homogeneous structure.

Fifteen cats were irradiated at the stereotaxic coordinates frontal 7, lateral 10, vertical 2. Lesions were made in both hemispheres of the brain using the following ultrasonic dosage: 50 pulses of 0.4 second duration, applied at the rate of one per second and at a focal intensity (corrected for absorption) of 600 watts per cm.\(^2\). The cats received an injection of trypan blue 45 minutes to 1 hour after the irradiation and were sacrificed and perfused 10 minutes later. After additional fixation in formaldehyde of about 5 hours the brains were cut in the appropriate frontal plane. Colored photographs were taken of each specimen.

Evaluation of the placement of the lesion was carried out as follows: each color film was projected onto a blown-up map of the frontal F-7 plane, and the projected outline of both the brain and the ventricles was matched as well as possible with the corresponding landmarks of the map. The center of the blue-stained cross-sectional area of each lesion was then marked on the map for each cat. The locations of the 29 "hits" as they have been recorded on the map are shown in Text-fig. 10.

For a study of the distribution of the "hits" about the target the combined results from both hemispheres have been projected into a single coordinate system centered in F-7, L-10, V-2. From the distribution of "hits" shown in Text-fig. 11 one immediately recognizes that the mean deviation from the desired lateral coordinates does not exceed more than ±1 mm. A similar distribution has been found to apply to the frontal coordinates. However, the majority of hits is located above the target, as may be seen from Text-fig. 12 which shows a plot of the vertical distribution. In this study of 15 cats, 80 per cent of the hits were located above the center of the lateral geniculate body. This result cannot be explained by an upward shift of the ultrasonic focal

\(^7\) See reference 7. These authors compare the size and severity of lesions on a relative basis, with special regard to the time rate of development of the postirradiative tissue changes.
Text-Fig. 10. Map of section located at frontal 7 showing results obtained with a group of 15 cats.
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Text-Fig. 11. Projected distribution of lesion centers around target (lateral geniculate body.)

Text-Fig. 12. Vertical distribution of lesion centers, as obtained from Text-fig. 11.

point: in a number of irradiations the location of the focus was checked by stereotaxic insertion of a small sound-sensitive probe into the center of the geniculate and was found to coincide with the desired coordinates.

We are thus led to conclude that the lateral geniculate body is more resistant to ultrasound than the fiber tracts surrounding it. Referring again to Text-
fig. 2, it is obvious that with a high damage threshold near the focus and a low threshold above and below the focus, the less resistant tissues adjacent to the geniculate body will be primarily affected. An improvement of this situation would require focusing systems of much larger intensity magnification at the focal point. We have tried multiple exposures with a cross-firing technique in which the angle of beam incidence was varied. The target was thus subjected to longer irradiation times than most of the surrounding white matter. However, so far no complete success has been achieved in destroying selectively the lateral geniculate body.

It is perhaps significant that the lateral geniculate body which we have found to be so extremely resistant to ultrasonically induced damage is one of the most vascular regions of the cat's brain (14). The relatively large volume of blood flow through this region would thus tend to carry away ultrasonically produced heat more rapidly than in the surrounding tissues. This cooling effect would prevent the tissue temperature rise to levels at which the destructive effects of the ultrasound are potentiated (see Text-fig. 6).

These results, taken together with the work of others (7), have led us to formulate the following hypothesis: based on differing anatomical characteristics, three "target situations" can be described which offer increasing difficulty with reference to the accurate placement of an ultrasonic lesion within the cat's brain. These are: (a) target in white matter, surrounded by grey matter (example: mammillo-thalamic tract); (b) target in white matter, surrounded by white matter (example: internal capsule), or, target in grey matter, surrounded by grey matter (example: midregion of caudate nucleus); (c) target in grey matter, surrounded by white matter (example: lateral geniculate body).

The increase in difficulty from (a) to (c) would appear to be a result of difference in susceptibility to the ultrasonic damage; fiber tracts are easily damaged while aggregates of cell bodies are relatively resistant. It is apparent that the intrinsic accuracy of the method may be tested best under conditions corresponding to case (b). Any errors in dosage or positioning are greatly reduced in case (a), whereas in case (c) even without error in the physical parameters misplacement of the lesion may occur.

If our hypothesis is correct the choice of the lateral geniculate body (grey surrounded by white) for our first target study would appear to have been less than ideal.

A further complication of this problem of accurate focal destruction is the fact that the loci of lesions placed by stereotaxic means may vary because of

In an earlier publication (3) we calculated the maximum possible focusing gain which depends on the depth of the target below the surface of the brain, on the ultrasonic frequency and on the total solid angle of irradiation. For a solid angle of $0.13\pi$, as used by us as well as by other investigators (see reference 1) and for a tissue depth of about 15 mm., corresponding to the geniculate the maximal gain is about 120 at 1 mcps. and about 450 at 2.5 mcps. The optimal frequency for this depth would be 5 mcps with a focal gain of about 910.
factors that have nothing to do with the ultrasonic method itself. The coordinates of the stereotaxic atlas of the diencephalon of the cat used by us are accurate only within ±1 mm., provided that normal material is used. No selection of the cats used in our studies was made: they were of both sexes, varied in weight between 4.5 and 9.5 lbs. and were in various degrees of health. Their body temperature, measured rectally, varied between 35 and 38°C. and their depth of anesthesia was not uniform. The position of the head holder, in particular the penetration of the ear bars was also subject to some variability although x-ray checks were made frequently to ensure proper positioning.

Secondary Lesions.—In the course of these target studies we observed that under certain conditions of dosage and geometry circumscribed lesions occurred distal to the focus, at the point of interception of the beam axis by the base of the brain. Such secondary lesions were only found at or near the ventral aspect of the temporal lobe where the diverging beam encounters the concave surface of the middle cranial fossa.

This particular location, and the absence of secondary lesions at other locations, even when the target was relatively close to bone, supports our view that "echo lesions" are caused by reflection of ultrasound at concave bone surfaces, with subsequent refocusing back into the cortex of the ventral lobe. Selective heating at the brain-bone interface may contribute to the formation of the lesion, but there is evidence that echo lesions cannot exclusively be ascribed to bone heating. For example, Fig. 7 shows an echo lesion after irradiation of the lateral geniculate body, in which the cortical grey matter is left intact in a small band adjacent to the bone. We would expect that echo lesions are unlikely to occur under conditions, such as in many regions of the human brain, in which reflecting bone surfaces are far away from the target; however, this view requires further confirmation.

SUMMARY AND CONCLUSIONS

Ultrasound at frequencies of 1.0 mcps and 2.5 mcps can be focused by a suitable lens system to produce a small region of high vibrational intensity. The concentrated energy within and around the focal region can be used to destroy structures of the central nervous system.

The extent of destruction depends upon: (a) the size of the focal region, which varies inversely with the frequency, (b) the ultrasonic intensity, (c) the duration of exposure, and (d) the physical and physiologic characteristics of the tissue under irradiation.

With proper choice of ultrasonic dosage, mice were rendered monoplegic by destruction of one-half of the spinal cord without demonstrable injury to the skin or subcutaneous tissues through which the converging ultrasonic beam had been transmitted. In similar fashion, focal lesions were produced in the basal ganglia of living cats by stereotaxic transdural application of a focused ultrasonic beam delivered through the superior aspect of the cerebral hemispheres.
Histologic studies of mouse spinal cords and cat brains offered evidence that the fiber tracts of the central nervous system are more vulnerable to ultrasonic irradiation than aggregates of cell nuclei or vascular structures.

The destructive action of the ultrasound is apparently a result of mechanical strain combined with a rise in temperature at the focus of the beam. The heating factor was found to assume greater importance under conditions of high intensity and continuous (rather than pulsed) irradiation.

Trypan blue staining and radioautography using P32 have been employed to identify the lesions 1 hour after irradiation. This has been a valuable adjunct in our attempts to determine the accuracy of placement of the lesions and their size. Perhaps more important, however, is the indication from these studies that ultrasonically produced lesions may offer a useful method for investigation of the nature of the blood-brain barrier.

“Target studies” were undertaken to determine the precision with which lesions of predetermined size could be placed at predetermined sites in the basal ganglia of the cat. Results to date have been promising, but it is our opinion that further technical improvement will be necessary before ultrasound can be used as an accurate method for placing discrete lesions within the human brain.

BIBLIOGRAPHY


10. Hueter, T. F., Messung der Ultraschall Absorption im menschlichen Schadelkno-
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EXPLANATION OF PLATES

PLATE 28

Fig. 1. Spinal cord of a mouse rendered immediately paraplegic by ultrasonic radiation. The specimen was obtained 6 hours after irradiation. The section shows intensive disintegration of the spinal cord at the irradiated level. The central region contains only a few "ghosts" of nerve cell bodies; the white matter displays a markedly homogeneous eosinophilic structure, in which only scattered filaments can be recognized. By contrast, numerous apparently normal glia nuclei are present, chiefly of the oligodendroglia type. The pial covering and the spinal nerve roots appear normal. Apart from diffuse local extravasations of erythrocytes no hemorrhage has occurred. X 130.

Fig. 2. Section of the spinal cord of a monoplegic mouse, showing the zone of maximal injury. The left half of the cord has been almost completely severed at this level, while the right side shows injury to the dorsal horn, dorsal funiculus, and dorsal half of the lateral funiculus. The integrity of the right ventral quadrant of the cord (containing, among other fiber systems, reticulospinal and vestibulospinal tracts) must have accounted for the absence of paralysis in the right hind limb. X 120.
Ballantine et al.: Nervous tissue destroyed by focused ultrasound
PLATE 29

Fig. 3. Spinal cord section of a paraplegic mouse a slight distance above the site of maximal injury. Note confinement of the damage to the dorsal funiculi and to the more heavily myelinated fiber systems of the lateral funiculi which appear partially disrupted. The grey matter is almost entirely intact at this level. X 105.

Fig. 4. Vertical section of Cat 6. Right: Intensity 710 watts per cm², 30 pulses, Thionin stain. Left: Intensity 950 watts per cm², 30 pulses, Weil stain. X 3.
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PI~ATE 30

Fig. 5. Vertical section of cat 3–6, Weil stain. The center of the lesion traverses the internal capsule and optic tract. × 3.5.

Fig. 6. Black and white print of color slide obtained from gross brain section of cat 6–8; the lesions stained by trypan blue, involve both lateral geniculate bodies. × 4.5.

Fig. 7. Secondary lesion near ventral aspect of temporal lobe. × 7.
(Ballantine et al.: Nervous tissue destroyed by focused ultrasound)