

EFFECTS OF HIGH INTENSITY ULTRASOUND ON
THE CENTRAL NERVOUS SYSTEM
OF THE CAT¹

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TWENTY-EIGHT FIGURES

The effects of low intensity ultrasound on various tissues of the body have been extensively studied by European investigators (see Matthes and Rech, '49). American workers such as Lehmann ('53) and Herrick ('53) also have used low intensities and these latter authors conclude that the changes produced in the tissues are caused by the heating effects of the sound. Fry ('53) and his co-workers have used high intensity ultrasound in their work. The present paper is a continuation of these later studies, and is an attempt to show histologically the effects of intense ultrasound on the central nervous system.

Earlier histological observations (Wall et al., '51, '53) obtained on animals irradiated at this laboratory indicated that the cell bodies were more susceptible than the fibers. These preliminary results have not been substantiated by further, more detailed studies. In fact the results reported herein show conclusively that the white matter is more readily affected by the ultrasound than the gray matter.

METHODS

Experiments on 104 cats constitute the basis of this report. The cats were anaesthetized with sodium pentobarbital and inserted into a stereotaxic head holder. The calvarium was

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removed bilaterally and symmetrically even though the ultrasound was administered only to one side. Sterile techniques were used throughout the entire procedure. The dura was left intact although occasionally when a small tear was made in it no difference could be seen in the results. The skin edges from the longitudinal midline incision were pulled up over

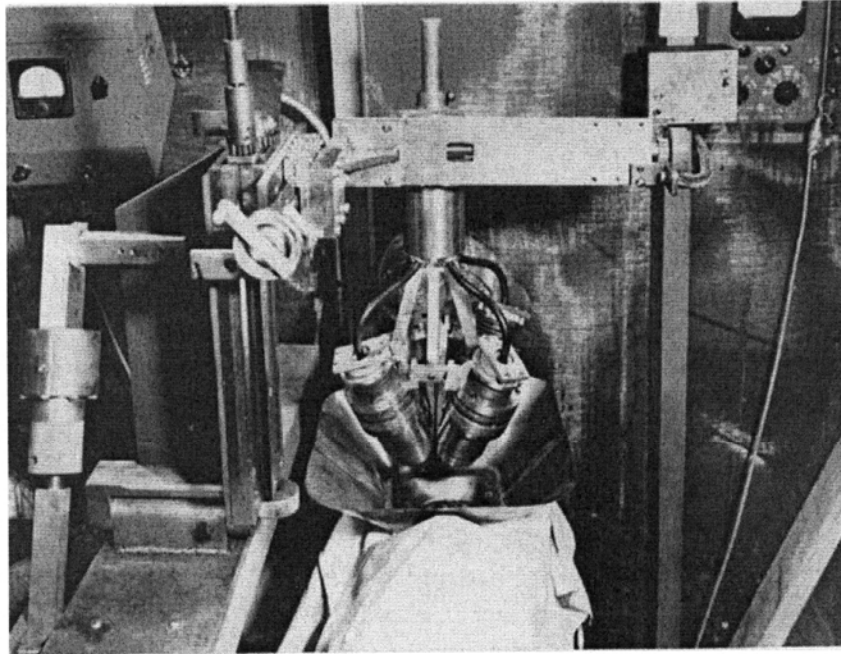


Fig. 1 The animal is mounted in a head holder with the pan tied to scalp. The multiple beam acoustic transducer is supported by a stereotaxic instrument. Ringer fluid has not yet been inserted.

the lower ring-like edge of a large pan which was positioned over the head. A metal wire pulled snugly around this edge gave a water tight connection between the cat's head and the pan (fig. 1). Before irradiation this pan was filled with sterile, warm, degassed Ringer solution (no carbonate was put in the Ringer) and the crystal transducer of the sound head was placed in it so that there was a liquid continuum between the source of the sound and its focal point in the brain.

The sound head was previously oriented stereotaxically to the head holder and therefore could be moved along three axes exactly like an electrode in the conventional stereotaxic instruments (fig. 1).

To keep the most intense region of the ultrasound field small in size two basic types of acoustic transducers were used (fig. 2). Detailed characteristics and design features of these transducers will be published elsewhere (Fry and Fry, '56). Each focused the sound energy into a narrow beam whose width and length, as measured between the points where its intensity had fallen off 50% was 1.5 mm wide and 10 mm long for the multibeam instrument (fig. 2 A) and 1.5 by 13 mm for the parabolic reflector (fig. 2 B). This type of parabolic reflector was first suggested by T. F. Hueter. The shape, size and depth of focus of both these beams were found unchanged whether the beams travelled through Ringer solution or through 3-4 cm of cat brain. This follows from the fact that the velocity of sound in Ringer solution is less than 2% different from that in brain tissue (Ludwig, '50).

The characteristics of the beam were measured at the center of the focus and differed for various experiments. This determination of the characteristics of the focused beam was made at frequent intervals between experiments by the method developed by Fry and Fry ('54). For all of the animals the frequency was kept at 980 kc per second. The wave length in the tissue is then about 1.5 mm. From a number of possible methods of procedure one was adopted which permitted the irradiation of a large area of brain. An array of spots was irradiated with 0.5 mm between centers, yielding an overlap so that all tissue was subjected to an intensity at least 0.9 of the peak value. One minute was allowed to elapse between each adjacent shot in a row. Since never less than 5 rows were used it was necessary to lay down one row of spots after another, the whole irradiation process occupying an hour or more. For internal capsule lesions the Horsley-Clark coordinates from anterior 9 to anterior 11 and laterally from 1 or 3.5 to 10.5 were covered with an array of spots 0.5 mm

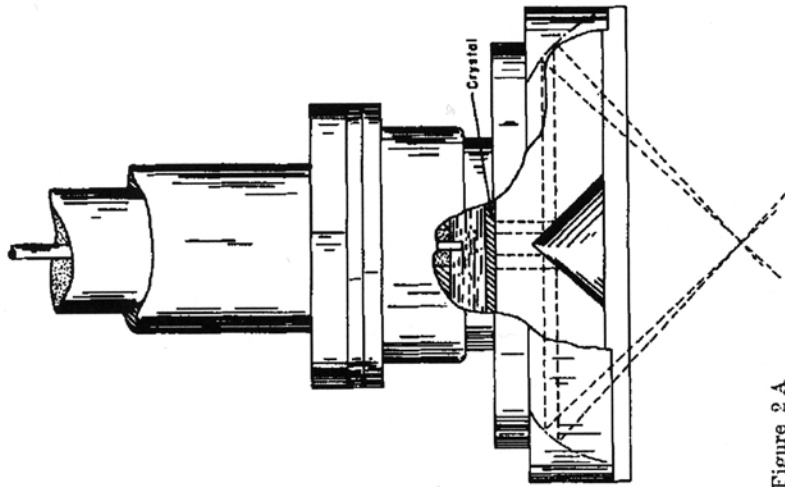


Figure 2 A

Fig. 2 A The parabolic acoustic transducer which produces a focused beam.

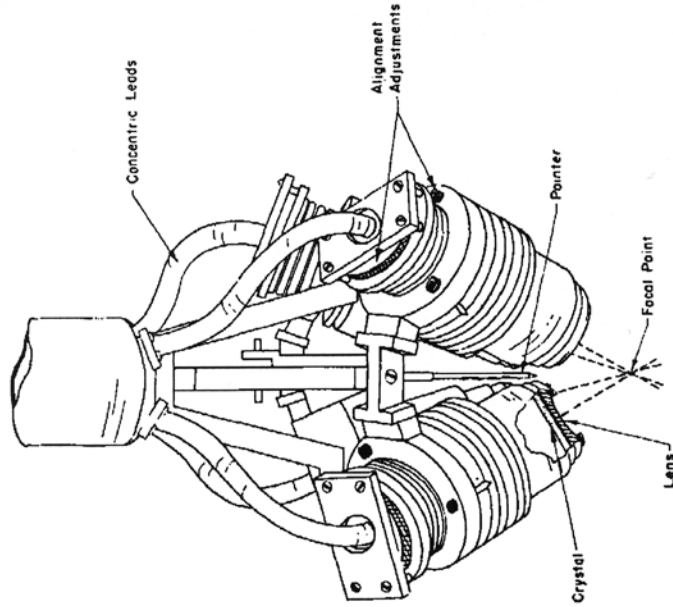


Figure 2 B

Fig. 2 B The multiple beam acoustic transducer consists of four separate focusing elements. The focused beams from all the elements intersect in a common region.

apart. The vertical level for all points was 7 mm above the horizontal plane through the ear bars. For the cortical lesions a series of spots were irradiated which extended 10 mm out from the midline over the lateral and suprasylvian gyri. The beam was focused 3 mm below the surface at the midline. As the beam swept laterally this depth decreased as the brain surface curved ventrally. Caudorostrally the rows of points extended 5 mm, and were roughly centered in the area delimited by medial extensions of the anterior and posterior ectosylvian sulci.

After irradiation, the pan was removed and the scalp layers and skin were sutured. The animals were given penicillin intramuscularly. The internal capsule cats were tested daily for defects in posture, gait, and reflexes. Animals were sacrificed at 2, 6, 12, and 24 hours and at 2, 3, 4, 12, and 30 days postoperatively under sodium pentobarbital. They then were bled and their brains were perfused with or immersed in 10% formalin. The pertinent parts of the brains were imbedded in celloidin or paraffin and sectioned at 30 or 15 μ respectively. A series of every 5th section was stained in thionin (Windle) and another series in Weil's myelin stain. Sample paraffin sections were stained in Romanes silver. Marchi preparations of the medulla and spinal cord were made in many instances.

In most of the earlier cats done in this series, the animals were sacrificed in the stereotaxic machine and pins were inserted in the brain at positions such that the area irradiated would be clearly marked. After large, clear lesions were consistently obtained this practice was omitted from the procedure because it was unnecessary. A vertical pin hole may be seen in figure 28 for example.

RESULTS

Early in this work it was found that the irradiation of tissue close to bone caused brain damage that appeared adjacent to the bone. This is reasonable in view of the high ultrasonic absorption coefficient of bone. At high intensities

the time rate of heat production in bone is very large and measurements of the resulting temperature change indicate that it is sufficient to burn the tissue with the dosage conditions used in these studies. Histologically this has been confirmed many times. The work of Lynn and Putnam ('44), wherein large lesions were made in the brain, is subject to this criticism, in that they irradiated with ultrasound through the intact skull. The heat generated in the bone and conducted to the brain tissue probably accounts for the lesions they obtained. A graded damage to brain tissue adjacent to bone can be produced by ultrasound of different intensities incident on the bone. Complete necrosis and sloughing is produced immediately next to the bone for the highest dosages used, milder tissue reactions of slower fiber and cell death and subsequent gliosis appear in response to intermediate exposures, and finally in positions farther from the bone only varying grades of edema occur. There is apparently no difference in the response of cells, fibers or blood vessels under such conditions. All are equally vulnerable to heat. Because of the factor of bone heating the work in this report is confined only to studies on the cerebral cortex and on the internal capsule where bone is sufficiently far away to eliminate any influence from that source.

Of the 104 cats studied only a few can be presented. These were selected on the basis of the reliability of the data and the fact that each type of lesion had been reproduced a number of times in different animals. Whether chosen from the group with irradiated cortices or from the series with irradiated internal capsules all specimens illustrate a consistent and progressively developing picture of the reaction of the brain to ultrasound. The lesions produced in all experimental animals conformed to the picture presented by the selected sample.

The following table lists the values for the acoustic variables and the duration of irradiation at each spot in the array, for the cats whose reaction is considered in detail. The estimated accuracy for the quantities which characterize the sound field,

in these particular experiments is $\pm 5\%$. The cats are listed in a sequence of increasing survival times and are described in that order.

Two hours after irradiation. Cat 163 was sacrificed two hours after the beginning of the one hour and 40 minute irradiation of its cerebral cortex. The lesion shows best in that area first irradiated, i.e., where the longest time had elapsed (2 hours) before sacrifice. The lesion under low power (fig. 3) appears as a light area in the dark myelin of the subcortical white matter. The cells of the cortex are

CAT NO.	PRESSURE AMPLITUDE	PARTICLE VELOCITY	DURATION OF SHOT	SURVIVAL TIME	REGION IRRADIATED
	atm	cm/sec	sec		
C163	23	200	4.00	2 hours	cortex
C147	23	200	4.00	6 hours	cortex
C162	23	200	4.00	12 hours	cortex
C150	23	200	4.00	1 day	cortex
C146	48	420	1.00	2 days	cortex
C148	23	200	4.00	2 days	cortex
C111	48	420	1.00	3 days	motor cortex
C101	24	190	4.00	3 days	int. capsule
C100	24	190	4.00	4 days	int. capsule
C131	30	260	4.00	4 days	int. capsule
C98	24	190	4.00	12 days	int. capsule
C154	23	200	4.00	12 days	cortex
C76	24	190	4.00	30 days	int. capsule
C80	22	170	7.00	32 days	int. capsule

intact and show no response to irradiation even in the sulci which were at the depth of the focused beam of ultrasound. Under higher powers (figs. 4 A, 5 A), the lesion in the white matter shows a great number of clear spaces especially around blood vessels. Almost all the myelin has taken the form of hollow spheres, which in section appear as myelin rings, there being only occasional short fragments of fibers running in the plane of the section. This is in contrast to the same area on the opposite side of the brain where many fibers run in the plane of the section and in fact dominate the pattern (figs. 4 B, 5 B). On the irradiated side only an oc-

casional longitudinally running fiber may be seen in myelin stained material. Close examination of such fibers reveals that they are swollen and varicose and end in a large ring of myelin. The axis cylinders are completely disrupted. In

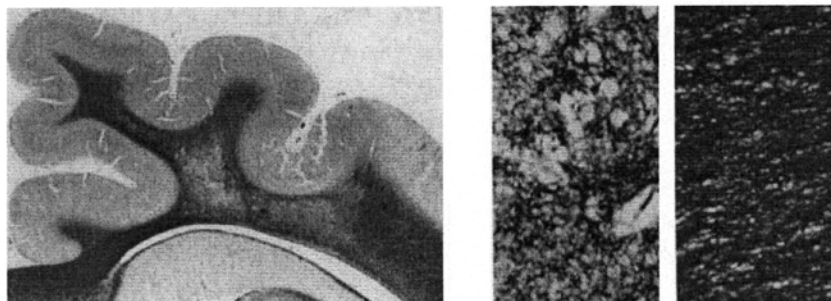


Figure 3

Figure 4 A

Figure 4 B

C-163 2 HOURS

Fig. 3 Two hours after exposure. Coronal section of lateral and medial suprasylvian gyri with the lesion underlying the latter. Weil stain; $\times 2.5$.

Fig. 4 A Two hours after exposure. Center of lesion showing myelin spheres. Weil stain; $\times 55$.

Fig. 4 B Normal white matter. Weil stain; $\times 55$.

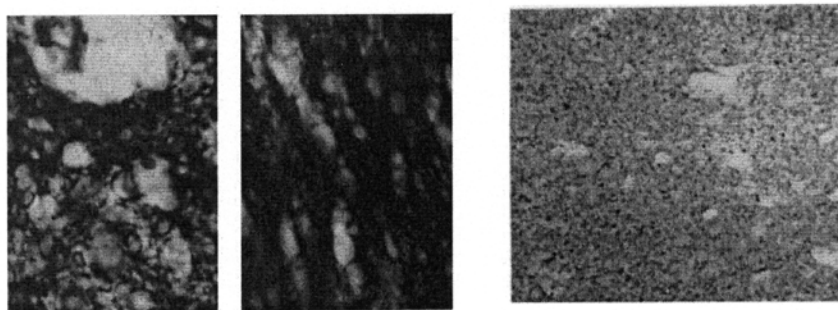


Figure 5 A

Figure 5 B

Figure 6

C-163 2 HOURS

Fig. 5 A Two hours after exposure. Center of lesion showing myelin spheres and enlarged perivascular space. Weil stain; $\times 330$.

Fig. 5 B Normal white matter. Weil stain; $\times 330$.

Fig. 6 Two hours after exposure. Absence of axis cylinders in the center of the lesion. Romanes; $\times 55$.

silver material only an occasional short bent remnant of a fiber is found (fig. 6). The glial meshwork is stretched out with the interstices filled with myelin rings, glial cells, and some detritus. There are in addition larger spaces which appear to have nothing in them. These are especially prominent around blood vessels, where they are undoubtedly distended perivascular spaces. This is probably part of the generalized edema. A gross view of the slide reveals that

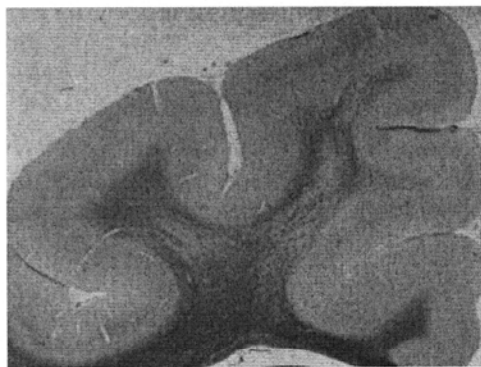


Figure 7

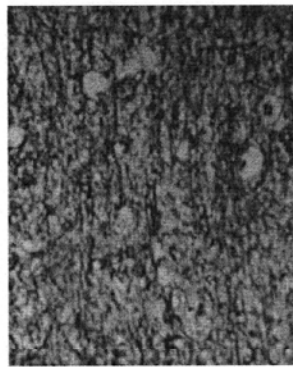


Figure 8

C-147 6 HOURS

Fig. 7 Six hours after exposure. Coronal section of irradiated gyri. Lesion is minimal. Weil; $\times 2.5$.

Fig. 8 Six hours after exposure. Myelinated fibers in the lesion are swollen and varicose but intact. Weil; $\times 55$.

the white matter in the irradiated region occupies about double the area taken by the same white matter on the opposite side of the brain. This would tend to establish edema as part of the response. Blood vessels have the same appearance on the two sides of the brain. There are larger perivascular spaces on the irradiated side. No hemorrhages of any kind are found. The nerve cell bodies are unaffected by irradiation except in the deeper layers of the cortex where they are a bit more widely separated by the encroaching edema.

Six hours after irradiation. The brain of cat 147 shows a mild lesion in the white matter below the cortex (fig. 7). This white matter is swollen so that the irradiated gyri are thicker than on the control side. The blood vessels and capillaries of the two sides appear equal in the degree of dilation and the same amount of blood is present in them. The myelinated fibers are less disturbed in this cat than in the two hour cat. The fibers, in myelin stained material, are somewhat swollen and show many varicosities, but most of them retain their integrity (fig. 8). The glial cells show no changes. In silver material (Romanes) many axis cylinders do not appear affected except that they are more widely separated from one another which might be caused by the edema. The cells of the deepest layers of the cerebral cortex adjacent to the affected area are more separated from one another, indicating that edema is present. The nerve cells themselves appear normal. The attempt, throughout these experiments, to work with doses of ultrasound near threshold suggests that slight variations in the experimental conditions may produce large differences in the reaction of the tissue. Thus the lesion in this 6 hour cat represents the nearest to an actual threshold condition obtained in the experiments performed and is included for that reason.

Twelve hours after irradiation. The brain of cat 162 shows a large lesion in the white matter underlying the cerebral cortex (fig. 9). The lesion is more nearly like that of cat 163 (2 hour) than like that of cat 147 (6 hour). At threshold levels small differences in sound intensity may make large differences in the effects on the tissues. In any event this 12 hour cat shows a breakdown of the myelinated fibers into myelin spheres which appear as rings in the sections (fig. 10). Other details agree with those described in the two hour cat (163).

One day after irradiation. Cat 150 was irradiated in the usual manner, i.e., 3 mm below the surface of the cerebral cortex. There is a great deal of swelling as seen under the microscope (fig. 11). The white matter shows a large necrotic

center in which the fibers are totally disrupted. Most of the myelin spheres are now broken down, only a few being left. The main material in the field seems to be detritus only loosely interconnected (fig. 12). Large spaces are present. Arterioles and capillaries are present in the same abundance

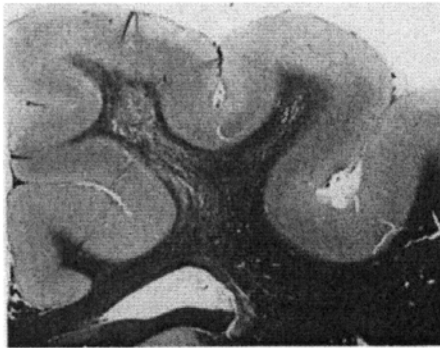


Figure 9

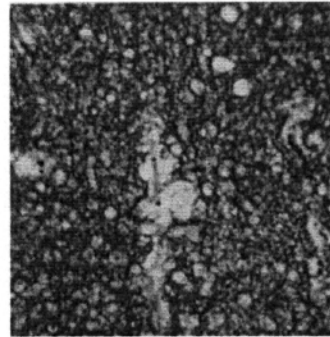


Figure 10

C-162 12 HOURS

Fig. 9 Twelve hours after exposure. Coronal section of irradiated gyri. Weil; $\times 2.5$.

Fig. 10 Twelve hours after exposure. Center of lesion showing myelin spheres and enlarged perivascular space. Weil; $\times 55$.

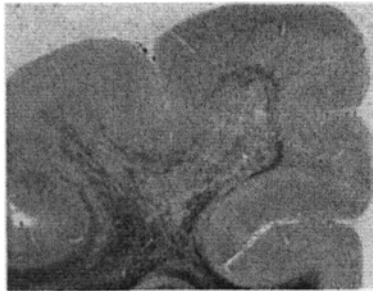


Figure 11

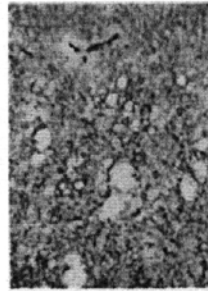


Figure 12

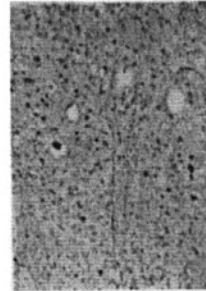


Figure 13

Fig. 11 One day after exposure. Coronal section of irradiated gyri. Weil; $\times 2.5$.

Fig. 12 One day after exposure. Myelin spheres, spaces, debris, and intact, blood filled vessels. Weil; $\times 55$.

Fig. 13 One day after exposure. Naked axis cylinders in area outside necrotic zone. Romanes; $\times 55$.

as on the normal side but with the difference that a few of the capillaries are broken and red cells are scattered in the tissue near the point of breakdown. The necrotic center is entirely confined to the white matter of the suprasylvian and lateral gyri. It is surrounded by an area of darker stain in the Weil and silver material. End bulbs of the retracting axis cylinders are found in this darker border zone. The myelin destruction extends beyond this zone out into the rays going into the cortical gray. In this outer region many naked axis cylinders are found (fig. 13). The space occupied by the myelin is swollen and vesicular. The myelin stains only weakly in the Weil stain as contrasted with comparable fibers on the normal side of the brain. The loci of the focal spots of the sound beam in the array of shots was a horizontal plane which passed through a great deal of cortical gray. The lateral sulcus bends down into the brain to the same depth as the lesion of the white matter extends and it was therefore as thoroughly swept by the irradiation as was the white matter inside the lateral gyrus on its medial aspect and the suprasylvian gyrus on its lateral aspect. The nerve cells of this irradiated gray are intact but in the deeper layers their perineuronal spaces are dilated and the cells appear to be indented or squeezed into angular shapes by this pressure. Examination of the cell material fails to reveal any hematogenous reaction at one day, i.e., leucocytes have not made their appearance in the tissues and the blood vessels have not thickened their walls. The glia in the necrotic center are spread far apart and stain only faintly.

Two days after irradiation. Cat 148 shows a large necrotic area (fig. 14) in the white matter underlying the middle suprasylvian and lateral gyri. The center is filled with the debris of myelinated fibers. The glial cells stain only faintly, presaging their death. The blood vessels in the necrotic center are now much fewer in number than they are in the first day material. At the edge of the necrotic zone the blood vessels are dilated and cuffing is becoming apparent. There are a few leucocytes present, the forerunners of the great hemato-

genous reaction which reaches its height at 3 and 4 days. This reaction zone surrounds the necrotic center on all sides. In Weil material it stains more darkly than the necrotic center and on high power inspection it is evident that the difference is not only in axis cylinder retraction bulbs but also in the blood vessels, leucocytes, and the glia. Just peripheral to the reaction zone, out in the medullary rays which go to the cortical surface of the gyri, the medullary sheaths of the nerve fibers are also destroyed but the glial cells and

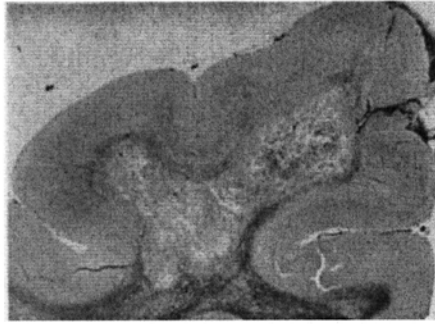


Figure 14

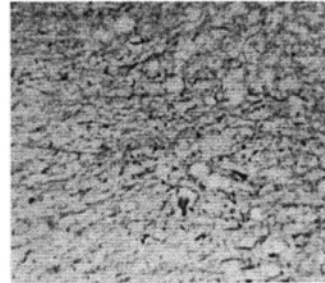


Figure 15

C-148 2 DAYS

Fig. 14 Two days after exposure. Coronal section of irradiated gyri. Extensive lesion. Weil; $\times 2.5$.

Fig. 15 Two days after exposure. Necrotic area on left, reaction zone in center, and peripheral zone with naked axis cylinders on right. Romanes; $\times 55$.

blood vessels are still intact. Many naked axons are present in this area which appear normal although without myelin (fig. 15). This makes these outlying areas stain more lightly in myelin and silver stains than normal white matter and even lighter than the reaction zone around the necrotic region but darker than the center itself. Normal myelin fibers are seen radiating out through the cortical layers. They seem almost as numerous and well stained as on the unirradiated side. The cortex of the middle suprasylvian and lateral gyri shows changes in the deep cell layers. Here there is obviously evidence of edema and cells are spaced farther apart.

The row like arrangement forced on the cells by the emerging fibers in normal cortex is broken up on the irradiated side. Certainly edema plays an important part in this reaction. The nerve cells of the irradiated cortex show many changes. In the deep layers a great number of the cells are surrounded by clear spaces which suggests that the perineuronal spaces of Virchow-Robin are dilated by the edema (fig. 16 A). The swelling of these spaces leads to distortion of the cell shapes. Normal cell bodies of the deep layer of the corresponding unirradiated cortex of the same cat are shown in figure 16 B.

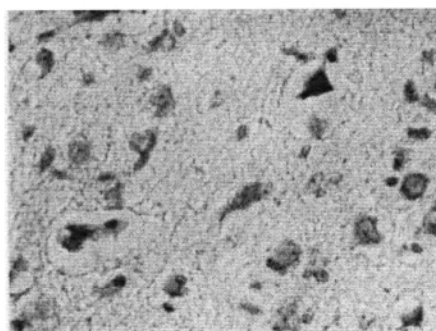


Figure 16 A

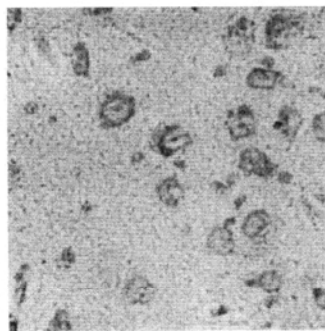


Figure 16 B

C-148 2 DAYS

Fig. 16 A Two days after exposure. Compressed nerve cell bodies in deep layer of the cortex. Perineuronal space. Thionin; $\times 130$.

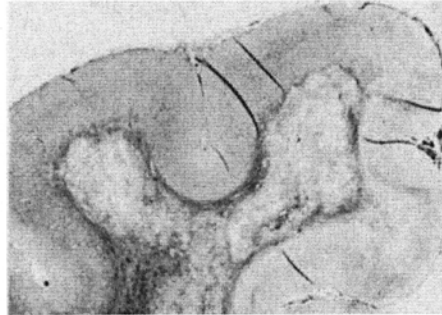
Fig. 16 B Normal cell bodies in deep layer of the cortex. Thionin; $\times 130$.

Closer to the surface of the cortex the cells gradually assume a more normal appearance. This is true even in the depths of the sulci which have been as thoroughly irradiated as the damaged and necrotic white matter.

Cat 146 is included in the series because it has a lesion very similar in quality and quantity to that found in the other two day cat (148). It is of interest because the temperature rise was approximately twice that of cat 148. The lesion in cat 146 (fig. 17) is almost identical in extent to that of cat 148 (fig. 14). The degree of destruction of the white matter is

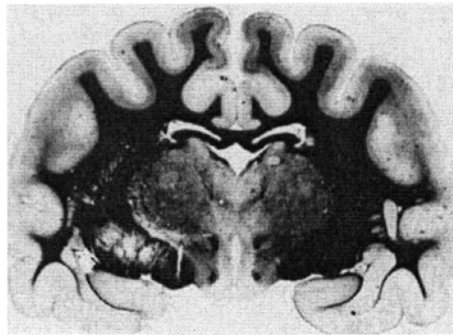
at the same level in both lesions. This is apparent in myelin, silver, and thionine stained material.

Three days after irradiation. Cat 101 has an internal capsule lesion (fig. 18). The forelimb on the side opposite



C-146 2 DAYS

Fig. 17 Two days after exposure. Coronal section of irradiated gyri. Extensive lesion, similar to that of figure 14. Weil; $\times 2.5$.

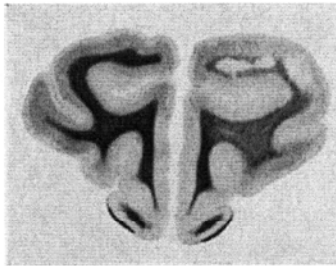


C-101

Fig. 18 Three days after exposure. Coronal section of brain. Lesion in internal capsula on left. Weil; $\times 1$.

the lesion was slow in visual and contact placing as compared with its opposite limb. The hindlimb opposite to the lesion showed only slow proprioceptive placing. The lesion itself extends approximately throughout the area irradiated. It shows a strong tendency to stay confined in the dense white

matter of the internal capsule and optic tract, although many cells of the ventral part of the reticular nucleus are destroyed. The lesion rises laterally as does the internal capsule. High power examination shows complete disorganization of the myelin and axis cylinders in its center. The oligodendroglial cells are mostly gone. Some polymorphonuclear cells and lymphocytes are scattered throughout the area. Many blood vessels seem to be lacking in the central region and some of those which are present show perivascular cuffing. There are other vessels which have blood elements in them but exhibit no perivascular cuffing. At the periphery of the lesion the vessels are numerous and dilated. Perivascular cuffing



C-111 3 DAYS

Fig. 19 Three days after exposure. Coronal section through motor area. Lesion on right side. Weil; $\times 1$.

is prominent. Polymorphonuclear cells are numerous and lymphocytes also are present. In addition to this hematogenous reaction the microglia are undergoing mitosis. Some of them are enlarged to the macrophage state. In this outer zone of the lesion there are neurons of the reticular nucleus in all stages of disruption from normal in all respects to fragments of neurons. Small hemorrhages can be found here which arise from capillaries and venules.

Cat 111 was irradiated in the motor cortex. The sound was focused on the upper lip of the cruciate sulcus. Hemorrhage occurred in the most lateral part of the cortex and here it is demonstrated that hemorrhage is indiscriminate in its de-

struction of white and gray matter. It is significant that this area of hemorrhage was near bone where heat production was high. The more medial area is like the irradiated zone in the midcortex of animals already discussed under the 1 and 2 days survival times. Central necrotic areas with edema but no hemorrhages are present. The actual pattern of the shots is reflected in the histology (fig. 19) in that the necrotic areas are 0.5 mm center to center (correction being made for shrinkage) with darker bands between them which represent some normal myelinated fibers which were spared by the ultra-

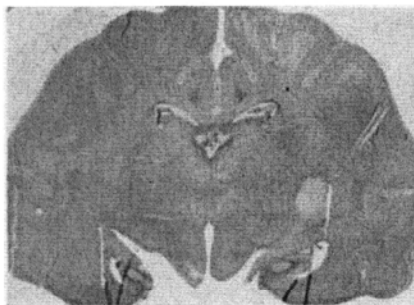


Figure 20 C-100

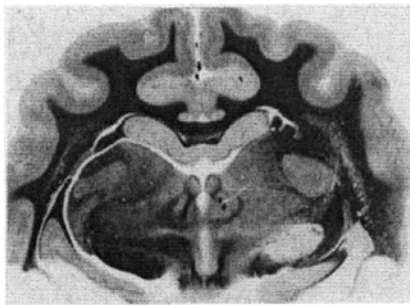


Figure 21 C-131
4 DAYS

Fig. 20 Four days after exposure. Coronal section with lesion in internal capsule on right. Thionin; $\times 1$.

Fig. 21 Four days after exposure. Coronal section with lesion in internal capsule on right. Weil; $\times 1$.

sound. Aside from this pattern the lesion is similar to that of other three day animals.

Four days after irradiation. Cat 100 showed no disturbance in gait or visual and contact placing at any time. This cat has a lesion (fig. 20) in the lateral part but none in the medial part of the internal capsule which is consistent with the lack of symptoms. The damaged area is restricted in great part to the white matter of the lateral part of the internal capsule and the optic tract with some encroachment on the reticular nucleus. The histopathology is similar to that described for the three day animal. There is however, a more

prounced ring of blood vessels with perivascular cuffing around the necrotic center.

Cat 131, also sacrificed after 4 days, showed no contact placing with the limbs on the side opposite the irradiation. The lesion (fig. 21) occupies a good part of the internal capsule and certainly encroaches on the forelimb and hindlimb area of the internal capsule (Barnard et al., '53). The destroyed area is similar to that in cat 100 with the addition that out in the healthy but reactive, marginal zone there are more of the small (100–200 μ in diameter) hemorrhages of arterioles. No

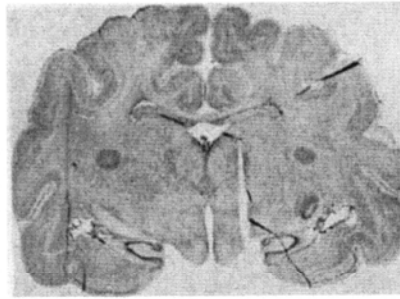


Figure 22 C-98

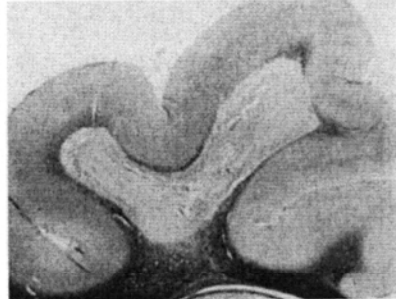


Figure 23 C-154

12 DAYS

Fig. 22 Twelve days after exposure. Coronal section with lesion in internal capsule on right. Thionin; $\times 1$.

Fig. 23 Twelve days after exposure. Coronal section of irradiated gyri. Weil; $\times 2.5$.

evidence of hemorrhage is present in the main part of the damaged area even with Weil stain which stains red blood cells strongly.

Twelve days after irradiation. Cat 98, irradiated in the internal capsule, showed only transient difficulty in contact placing of the opposite sided limbs. Only a small lesion (fig. 22) which is well confined to the internal capsule and optic tract is found. The hematogenous response has now disappeared and the macrophages show great activity. There are many thin walled vessels in the central necrotic region. These

may well be new vessels which have grown in since the lesion was made.

Cat 154 received a cortical irradiation 12 days before sacrifice. A large necrotic lesion is found occupying almost the entire white matter under the middle suprasylvian and lateral gyri. Blood vessels, debris, and debris-filled macrophages occupy the entire area (figs. 23 and 24). The adjacent cortex is no different from its fellow of the opposite side except that some of the large pyramidal cells of layer 5 are beginning to

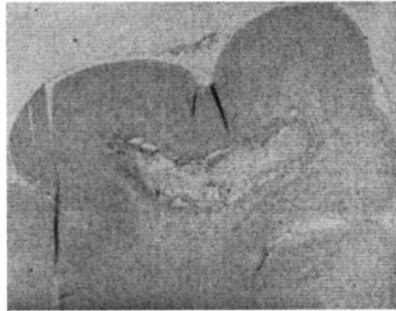


Figure 24

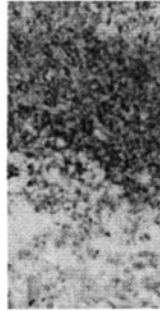


Figure 25

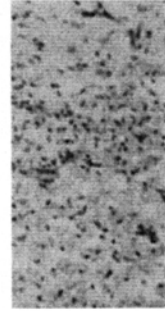


Figure 26

C-154 12 DAYS

Fig. 24 Twelve days after exposure. Coronal section of gyri near caudal end of irradiation. Thionin; $\times 2.5$.

Fig. 25 Twelve days after exposure. Cortex above, reaction zone (dark) middle and necrotic area below. Weil; $\times 55$.

Fig. 26 Twelve days after exposure. Cortex above, reaction zone middle and necrotic area below. Thionin; $\times 55$.

show early chromatolysis. This is probably a retrograde reaction to axon injury. Edema has disappeared and no chromatolysis or other abnormalities exist. The cell density of the cortex appears to be the same on both sides of the midline. Figures 25 and 26 show deep cortex, reaction zone and necrotic center.

Thirty days after irradiation. Cat 76 suffered a loss of visual and contact placing in the limbs on the side opposite to the lesion. It later recovered some measure of placing ability but it still was more difficult to elicit 30 days later than on the

normal side. There is a minimal lesion, confined to the center of the internal capsule (fig. 27). The rectangular array of spots was placed to cover both the internal capsule and the ventral posterolateral nucleus. The lesion stays well restricted to the white matter. Counts per unit area were made in the ventral posterolateral nuclei of both sides in 18 different sections such that on one side the area counted was always within the irradiated zone and its count was compared with that of a matched area on the opposite side. A careful examination of landmarks in the series revealed it to be almost

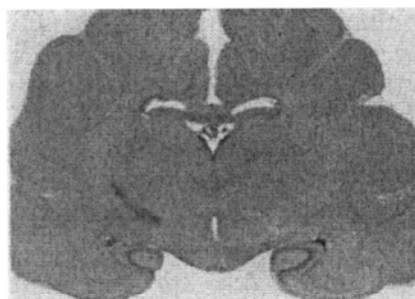


Figure 27 C-76

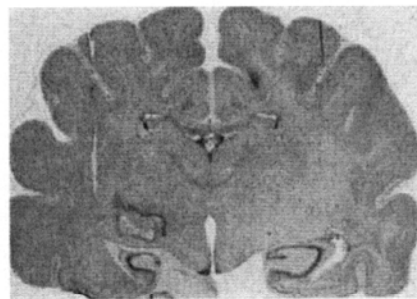


Figure 28 C-80

30 DAYS

Fig. 27 Thirty days after exposure. Coronal section with lesion in internal capsule on left. Thionin; $\times 1$.

Fig. 28 Thirty days after exposure. Coronal section with lesion in internal capsule on left. Thionin; $\times 1$.

exactly cross cut. A 12% reduction (1777 compared to 1569) in the number of cells was found on the irradiated side. This figure is not highly significant from the statistical point of view since control counts on non-irradiated cats varied as much. In addition to this possible lack of significance a contour map of the nucleus showed the irradiated nucleus to be somewhat larger (10%) than the normal nucleus over the same sections from which the counts were made. In short, the possible drop in the number of cells per unit area was compensated for by the same percentage increase in the size of the nucleus. To determine whether this difference in the size

of the nucleus was the result of irradiation or was a natural variation from side to side an area study was made of the lateral geniculate nucleus. One side was 2.8% smaller than the other. All of this would suggest that there was some difference in the two nuclei due to irradiation but that the difference was in the amount of intercellular space rather than in the number of cells. A further fact elicited from the material while making the unit area counts is that a larger number of pathological cells were found on the irradiated side than on the control side (50 compared to 18). The greater number of the pathological cells may well represent a retrograde response of the cells to damage to their axons out in the internal capsule where the obvious lesion is. Histologically the lesion is made up of many macrophages which appear to be starting their well known glial scar. That many nerve fibers have been destroyed is attested to by the Marchi material of the pyramid where the degenerating myelin is stained. A count of the Marchi granules gives 1887 on the lesion side and 28 on the normal control side.

Cat 80, sacrificed 32 days after irradiation, had contact and visual placing absent in the limbs opposite to the side of the lesion for its entire postoperative period. The lesion (fig. 28) is confined to the internal capsule and optic tract. Some encroachment on the thalamic reticular nucleus is apparent. This lesion is possibly destined for a fluid filled cyst condition instead of a dense scar as in cat 76. The glial response is like that of cat 76. Marchi granule counts in the pyramid give 2415 on the affected side and 20 on the normal side.

Heat measurements in brain tissue during ultrasonic irradiation. Constantan-copper (0.003" dia. wire) thermocouples were threaded into the brain of an anaesthetized cat. Using the method of short acoustic pulses (0.1 sec.) at low intensities compared to irradiation levels as detailed by Fry and Fry ('54), the thermocouple junction in the cat's brain was accurately located at the focal center of the sound beam. After locating the junction, a 4 second pulse of

ultrasound was delivered and the temperature elevation was recorded with a magnetic oscillograph. After a two minute interval the pressure amplitude was increased and another 4 second pulse delivered. This procedure was continued and a curve of peak temperature rise against the square of the pressure amplitude was obtained from the still living cats. Seven animals were utilized in this fashion, each with two thermocouples imbedded in its brain. Thus a family of curves was obtained. Each junction was carefully dissected out after the experiment and its position in the brain determined with special emphasis on its location in gray or white matter. It was found that for a pressure amplitude of 23 atmospheres for 4 seconds that the peak temperature rise in the white matter averaged 9°C. In the gray matter the values were about one-half this, i.e., the average rise was 5°C.

A further experiment, using the imbedded thermocouple technique, was done on freshly excised slabs of cat brain to measure the acoustic absorption coefficients of white matter and gray matter respectively in dead tissue. This was done to determine whether any readily detectable difference existed between living and freshly removed brain tissue in this respect. In this experiment the white matter exhibited an absorption coefficient twice that of the gray matter. This is in agreement with the *in vivo* studies, i.e., ultrasound is absorbed more by white matter than by gray matter in both living and freshly excised brain. This absorbed energy is reflected in the amount of heat produced. The possible significance of this is discussed later.

DISCUSSION

On the basis of our present studies it appears that high intensity, focused ultrasound at a frequency of 980 kc/sec produces changes in white matter of the central nervous system more readily than in gray matter. The first demonstrable effect is a swelling of the myelin and a tendency to show varicosities. The axis cylinders in a barely threshold effect do not appear injured (cat 147 — 6 hours). At the same time

there is an increase in the interstitial fluid and perivascular spaces. This process of damage to only the myelin may be reversible and further studies are planned. A more complete effect is that wherein the myelin and the axis cylinders are destroyed, the myelin assuming the form of hollow beads and the axis cylinders fragmenting (cat 149 — 2 hours and cat 150 — 24 hours). The interstitial and perivascular spaces are more evident at this level of injury than in the milder demyelinating process. The edema is great, but how much of it is caused primarily by ultrasound on vessel walls and other tissues or how much of it is secondary to the break-up of large amounts of axis cylinders and myelin is difficult to state. Possibly both factors exist. A study of smaller lesions may help to elucidate this problem.

Of outstanding interest to us is the relative resistance of gray matter to the action of ultrasound. However, in a severe reaction to a white matter necrosis with its attendant swelling the closely adjacent nuclei may be drawn into the breakdown area. This has occurred at times in the deep layers at the bottom of a cortical sulcus which overlies a necrotic center and it has happened to the parts of the reticular nucleus of the thalamus where these cells were close to a large necrotic center in the internal capsule. It is possible that different ultrasonic dosages might cause direct and permanent damage to gray matter. This will be investigated. (Please see addendum.)

The blood vascular system seems quite resistant to damage by ultrasound. It is conceivable that some of the edema which ensues comes from a changed permeability of capillaries. Edema does not explain the effects of almost instantaneous breakdown of the myelin and axis cylinders. Histological examination of many edematous cat brains reveals only a slow and mild response of the myelin. Similarly, vasoconstriction, or localized anemia cannot cause such great destruction in as short a time as one hour. Histologically the blood vessels look quite normal in the early stages of even a severe lesion. Full arterioles and capillaries as compared to constricted or empty vessels are found in about the same pro-

portion in both the irradiated area and its corresponding area of the opposite side of the brain. The only noticeable difference is in the perivascular spaces which are larger in the irradiated region. Only after 24 hours and increasing into the third day are there a few tiny hemorrhages which allow small numbers of red cells to escape. These occur mostly in the reaction zone surrounding the necrotic center and occasionally in the necrotic center itself. They appear to be part of the secondary reaction to the large area of necrosis. Small lesions, even though causing a small necrotic focus, do not show these hemorrhages.

As has been indicated previously acoustic energy absorbed in the brain causes heating. Heat will cause damage if the accompanying temperature change is sufficiently high and is maintained for a long enough period of time. Current work on frogs cooled to 1°C. shows that irreversible changes can be produced by ultrasound in the frog central nervous system in the absence of high temperature levels. The temperature of the frog nervous tissue during irradiation does not exceed 25°C. In the case of the cats reported herein the peak temperature rise in the white matter corresponding to the 4.00 sec. 23 atmosphere dose is 9°C. The gray matter had half this rise, 5°C. That the effects produced in the white matter by ultrasound and the increased susceptibility, to the sound, of white matter as compared with the gray are not simply a consequence of the temperatures reached, follows from a comparison of the effects of this dose with cats subjected to 48 atmospheres acoustic pressure for one sec. With this latter dose the gray matter had a 10°C. temperature rise and the white matter approximately a 20°C. rise yet the effect produced, extent and quality of the lesion in the white matter, and absence of effect on gray matter was similar to that of the 23 atmosphere dose.

SUMMARY

1. High level (20-50 atmospheres) focused, stereotaxically oriented, ultrasound with a frequency of 980 kc/sec is used to

irradiate the gray and white matter of superficial and deep regions of the cat brain.

2. The axons of white matter are readily susceptible to damage but gray matter, vascular tissue, and glia seem practically immune under the dosage conditions used in these studies.

3. Cats were sacrificed from 15 minutes to 30 days after irradiation. A description is given of the histological changes produced in the brains of these cats. Where the lesions involved the corticospinal tract the animal showed appropriate functional deficiencies.

4. A study of the temperature changes produced in gray and white matter during irradiation is reported.

5. Factors which might be involved in the mechanism of these effects in the white matter of the cat brain are discussed.

ACKNOWLEDGMENT

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ADDENDUM

In the interim since this paper was accepted for publication (September 1954) the ultrasonic dosage relations for producing gray matter lesions have been determined and reported (Fry et al., 1955 and Barnard et al., 1955).