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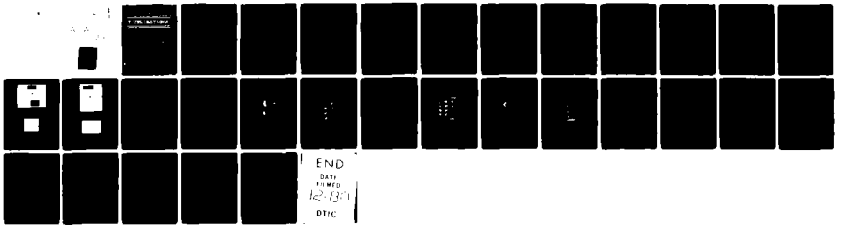

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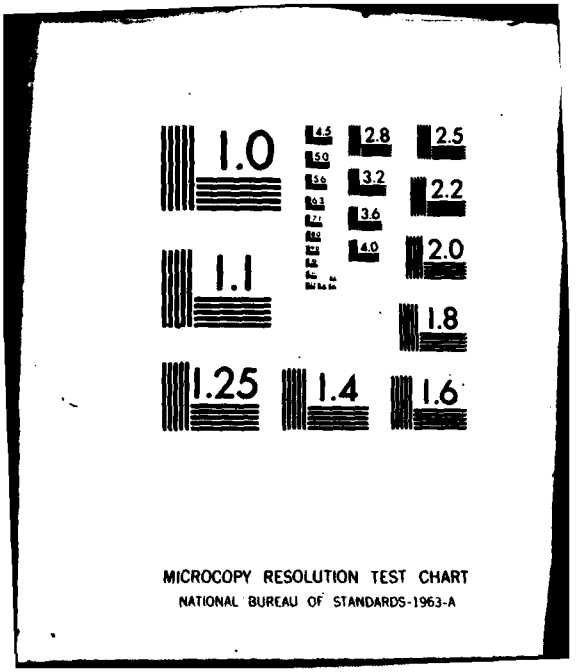
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Prepared For Office of Naval Research Physiology Program, Arlington, Virginia 22217

By James C. Lin, Ph.D.

Department of Electrical and Computer Engineering
Wayne State University
Detroit, Michigan 48202

CURRENT ADDRESS
Bioengineering Program
P.O. Box 4348
University of Illinois at Chicago Circle
Chicago, Illinois 60680

Contract No. N00014-78-C-0353
Work Unit No. 201-330

Scientific Report No. BES-4
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Final Report
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UIC-C

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James C. Lin, Ph.D.
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ABSTRACT

This investigation was designed to study the mechanisms of interaction between microwaves and the blood-brain barrier and was aimed at correlating changes of blood-brain barrier permeability with the quantity and distribution of absorbed microwave energy inside the brain of adult Wistar rats under sodium pentobarbital anesthesia. Through use of thermographic methods and a direct-contact applicator at the animal's head, the pattern of absorbed microwave energy was determined. Indwelling catheters were placed in the femoral vein. Evans blue in isotonic saline were used as a visual indicator of barrier permeation. Irradiation with pulsed 2450-MHz microwaves for 20 min at average power densities of 0.5 to 2600 mW/cm^2, which resulted in average specific absorption rates (SARs) of 0.04, to 200 mW/g in the brain, did not produce staining, except in regions that normally are highly permeable. When the incident power density was increased to 3000 mW/cm^2 (SAR of 240 mW/g), extravasation of Evans blue could be seen in the cortex, hippocampus and midbrain. The rectal temperature, as monitored by a copper-constantan thermocouple, showed a maximum increase of less then 1.0°C. The brain temperature recorded in a similar group of animals using a thick-film carbon thermistor exceeded 43°C. In one series of experiments, rats were irradiated at 3000 mW/cm^2 for 5, 10, 15 and 20 min. Immediately after irradiation all except the 5-min animals exhibited increased permeability in some regions of the brain. Brains of rats euthanized 30 min after irradiation were free of Evans blue, while those euthanized 10 and 20 min post-irradiation showed significant dye staining but with less intensity than those euthanized immediately after irradiation. These data suggest a total reversibility of microwave-induced blood-brain barrier changes within 30 min post-irradiation.

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INTRODUCTION

Although the effect of microwave irradiation on blood-brain barriers have been studied in laboratory animals, reports of the effects are varied and sometimes contradictory (1). For example, low levels of microwaves have been reported to increase cerebrovascular permeability (2,3), while other investigators, using purportedly the same experimental conditions, have reported no effects (4,5).

These apparent differences probably arise from the technical difficulties involved in the assay procedures and in determining the distribution of absorbed energy in a structure as complex as the head. In fact, measures of absorbed energy have not been reported to date in any archival study of the barrier's response to microwave irradiation (6). An irradiation procedure was developed that allowed a direct-contact applicator to be placed at a number of sites on the surface of the head. This method can limit the region of the brain undergoing irradiation and also lends itself to quantitative measure of the distribution of absorbed microwave energy.

Our study was designed to examine the mechanisms of interaction between microwaves and the blood-brain barriers, and to correlate any change in permeability with the quantity and distribution of absorbed microwave energy.

MATERIALS AND METHODS

Male Wistar rats (70-day old, 415-530 g) were anesthetized with sodium pentobarbital (40 mg/kg ip) and a plastic endotracheal tube was inserted to facilitate the animal's respiration. A polyethylene catheter was implanted in the femoral vein (PE 50). The animal was then placed prone over a polyfoam stand or body holder with the head elevated at an angle such that the top of the head was horizontal.

Rectangular pulses (10 μs, 500 PPS) of 2450-MHz microwave energy were delivered to the brain by a small dielectrically loaded coaxial applicator (Elmed, Addison, Ill.) in direct contact with the left side of the rat's head. The center of the applicator aperture (14 mm diam.) coincided with the intersection of the interaural line and a line just above the left eye. Thus, only the left hemisphere of the brain was irradiated. The averages and peaks of power density of incident radiation were calcualted from forward and reflected powers (see Figure 1), pulse width and pulse-repetition rate.

The distribution of absorbed microwave energy inside the head was determined by the thermographic procedure (using AGA thermovision) previously described by Guy (7). In brief: Two rats were killed and quickly frozen while in their holders. They were then cast in a polyfoam block and bisected either horizontally or parasagittally. The carcasses were then returned to room temperature. A thermographic baseline was taken over one-half of the carcass. The two halves were reassembled and irradiated for 15 sec at 20 watts by CW microwaves in the

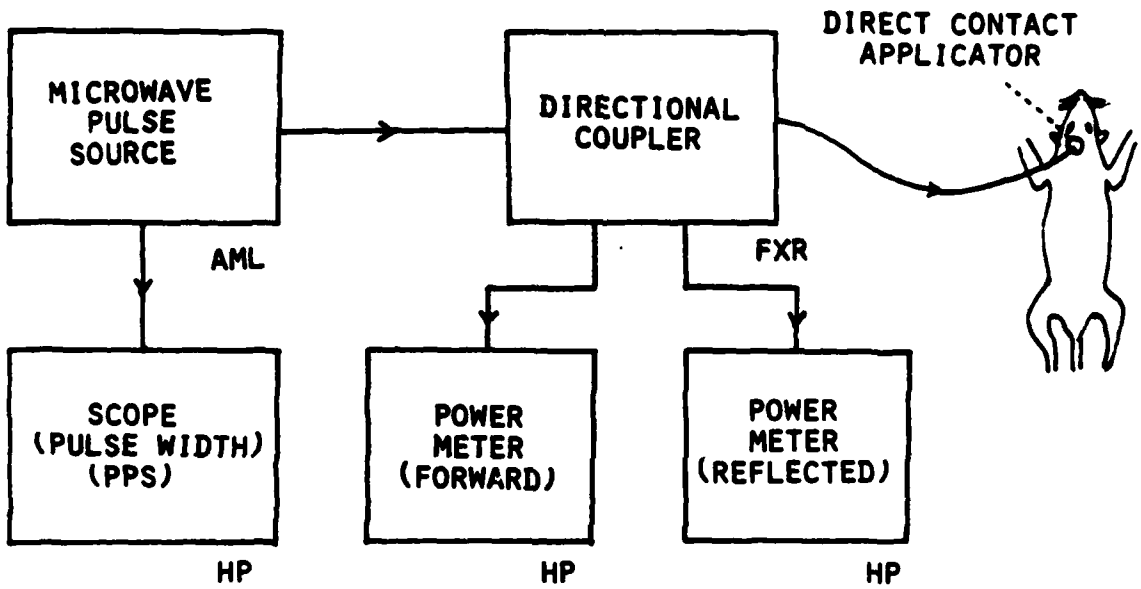


Figure 1. Microwave instrumentation for irradiation and monitoring.

same manner as before. A second thermographic scan was then made over the same half of the carcass immediately after microwave irradiation. The temperature increases were used to calculate specific absorption rates (SAR) by the equation

$$SAR(mW/g) = 4186\ c\Delta T/t$$

where $c = 0.88$ cal/g°C is the specific heat, ΔT is the increase of temperature, and t is the duration of irradiation in seconds.

The brain regions involved in microwave absorption were determined using a series of thermograms in which the areas occupied by the rat's head and brain were outlined. In addition, regions with enhanced energy absorption were further documented by superimposition of photographs of horizontal and parasagittal sections on the thermographs.

The rectal temperature was monitored using a copper-constantan thermocouple and digital indicator combination (Doric). Temperature in the cerebral cortex (at a point 3.4 mm to the left of midline, 2.7 mm ventral to the top of the skull and just anterior to the interaural line) was continuously monitored in a separate but similar group of 15 rats through the use of an indwelling thick-film carbon thermistor probe (Vitek Electrothermia Monitor, Boulder, Colo.). Following each experiment, the brain was removed and histologically observed to confirm placement of the probe.

A visual tracer, Evans blue (2 ml/kg of 2% solution) was injected into a catheterized femoral vein following sham or microwave irradiation. In one series of experiments (17 animals),

the incident power density was kept constant at 3.0 W/cm^2 while the irradiation duration was varied from 5 to 20 min. Evans blue dye was injected immediately following sham or microwave irradiation. In another series of experiments, rats (13 animals) were irradiated for 20 min at average power densities ranging from 1.0 to 3.5 W/cm^2 [A previous study (8) had indicated that irradiation at 0.5 to 1000 mW/cm^2 did not produce any increase in barrier permeation]. Evans blue dye was injected immediately following sham or microwave irradiation. In a third series of experiments, 16 animals were irradiated for 10 min at 3.0 W/cm^2. The visual tracer was injected 5, 10, 20 and 30 min post-irradiation in order to ascertain the reversibility of microwave-induced blood-brain barrier permeation.

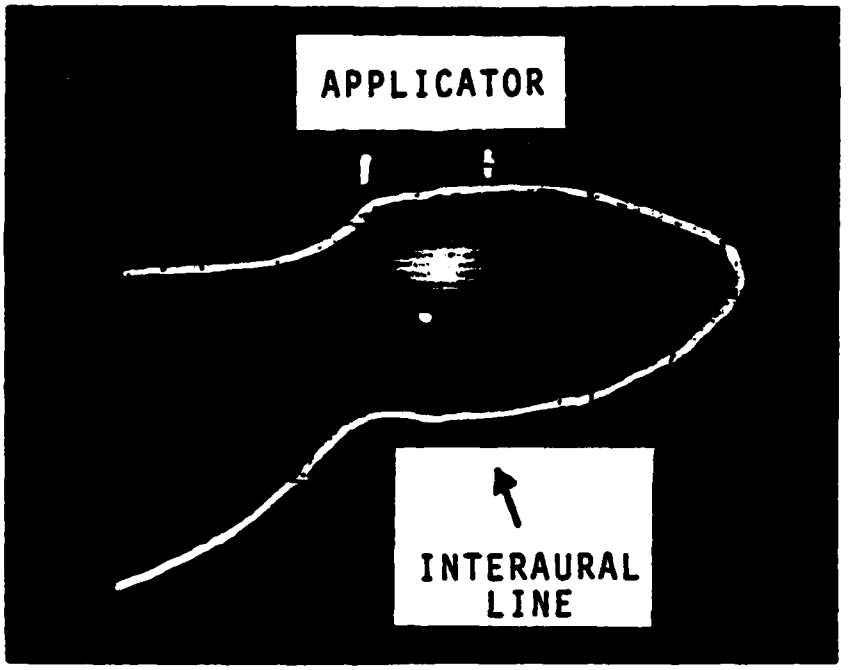
Each animal was perfused with normal saline via the ascending aorta five min after Evans blue injection. When the eyes and the forepaws were clear of pigment the animal was then fixed with 10% buffered formalin. The brain was removed and observed to ascertain gross cerebral regions in which the barrier might have been altered. The brain was left in fixative for evaluation at a later time.

During the evaluation stage the degree of tissue staining was independantly graded by two investigators on a scale of 0 to 6 according to the intensity and extent of staining. Subsequent to gross examination, 1 mm sections were cut and observed with light microscopy. The results were statistically evaluated using the Student's t-test.

RESULTS

Figure 2 illustrates specific absorption rates (SAR) over a horizontal section of a rat's head. The upper thermogram (Figure 2a) is an intensity scan in which brightness is proportional to SAR. It is seen that microwave energy was largely deposited in the left hemisphere. The lower picture (Figure 2b) is a line scan through the cortical region which was brightest on the intensity scan. The SAR distribution is fairly symmetrical at this plane (above the ears) and has a peak value of 39 mW/g per watt of delivered energy. Figure 3 displays the parasagittal distribution of abosrbed energy. Regions with enhanced absorption include thalamus, hippocampus, parietal and occipital cortex. The highest absorption occured in the cortical region, which has a peak SAR of 55 mW/g per watt of energy delivered. This difference in peak SAR is due to a more distal location of the horizontal section from the contact applicator. The higher SAR corresponds to a value of 0.08 mW/g per mW/cm^2 of incident radiation on the head.

The dependence of microwave-induced blood-brain barrier permeation by Evans blue dye on irradiation duration is summarized in Figure 4. The sham irradiated brains did not exhibit Evans blue staining, except in the pineal body, pituitary gland and the choroid plexus, regions in which cappillaries are known to be leaky. Five minutes of irradiation at a time-averaged power density of incident radiation of 3.0 W/cm^2 (time-averaged rates of energy absorption of 240 mW/g in the rat brains) produced



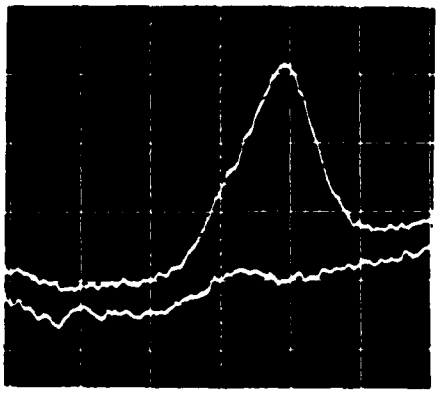
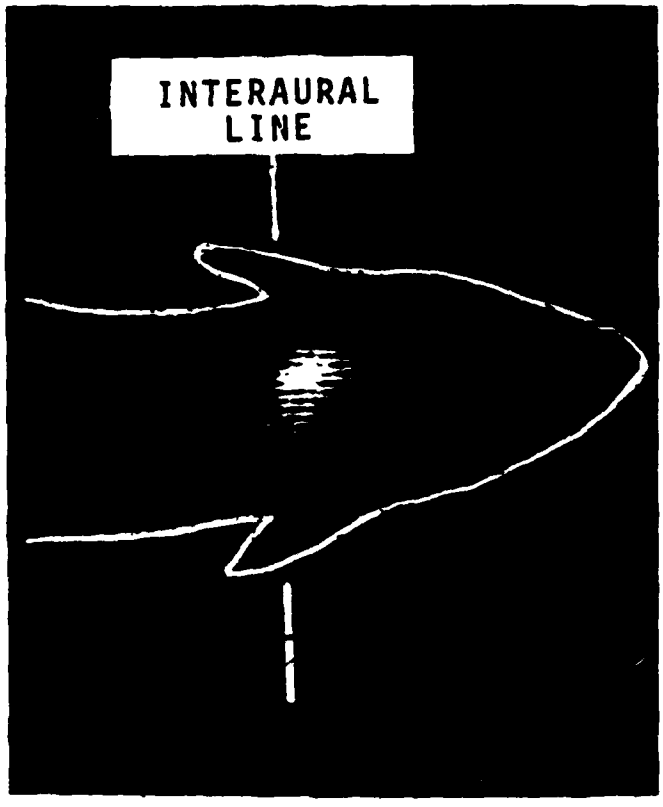


Figure 2. Distribution of absorbed microwave energy in a horizontal section of a rat's head after irradiation by 2450-MHz microwaves. The input power was one watt which results in a peak SAR of 39 mW/g. Temperature scale = 2°C/division; spatial scale = 1 cm/division.



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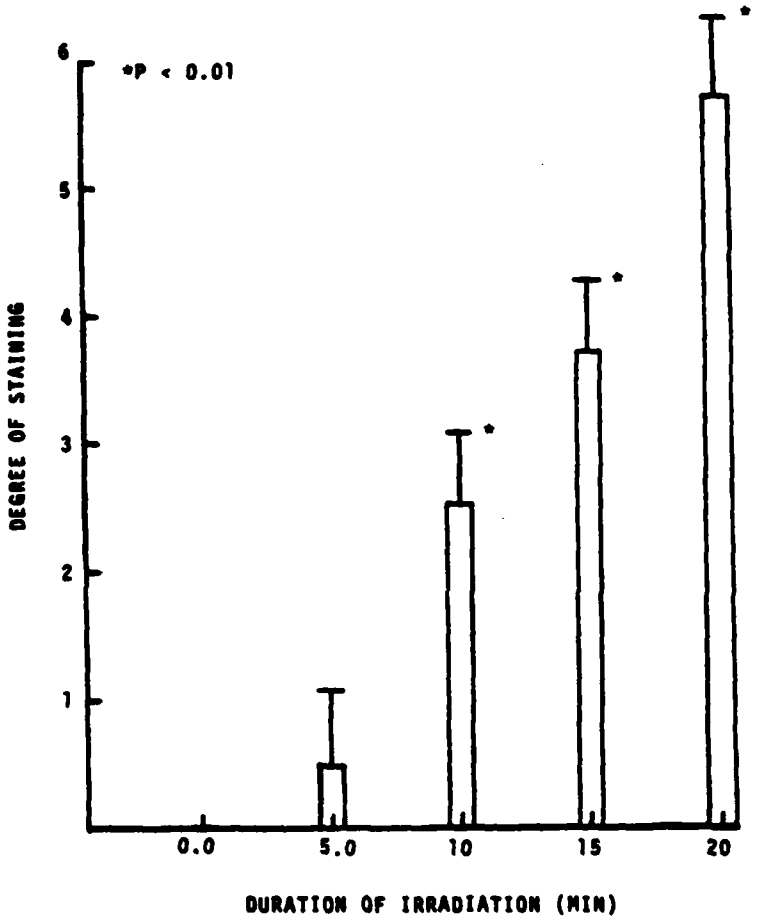

Figure 4. Degree of Evans blue staining of brain tissue following 5, 10, 15 and 20 min of 2450-MHz irradiation at 3 W/cm^2 (SAR of 240 mW/g). Each point represents the mean and standard deviation from mean of 3 or 4 animals.

slight cortical staining in some animals in addition to regions that normally are permeable (Figure 5). The staining was neither consistent nor statistically significant from the sham-irradiated animals ($p>0.1$). When the duration of irradiation was increased to 10 min and beyond, extravasation of Evan blue could be seen in the cortex, hippocampus and midbrain of rats killed immediately after irradiation (Figure 6). The extent of dye penetration into tissues and degree of staining were significantly different from that of sham-irradiated brains ($p<0.01$).

Figure 7 illustrates the relation of incident power density to microwave-induced barrier permeation by the visual tracer. It can be seen that time-averaged power densities less than 3.0 W/cm^2 failed to elicit any increment in the barrier's permeability when the incident power density was increased to 3.0 W/cm^2 and above, transgression of Evans blue through the blood-brain barrier became apparent immediately following irradiation. Unilateral hemispheric staining clearly visible in elements of the cerebral cortex, hippocampus, caudate nucleus and the thalamus (Figure 8).

Brains of rats killed 30 min after irradiation were free of dye staining (Figure 9). Animals euthanized 10 and 20 min post-irradiation showed considerable dye penetration into brain tissues but with less intensity than those euthanized immediately or 5 min after irradiation (Figure 10). These results are summarized in Figure 11. The monotonic reduction in Evans blue staining at 10, 20 and 30 min post-irradiation was significant at the 0.01 level.

Figure 5. Barrier opening at the left hemisphere of the rat by microwave irradiation (3 W/cm^2). Intravenous Evans blue was used as a barrier tracer. Irradiation periods: 311-20 min; 304-15 min; 306-10 min; 324-5 min.

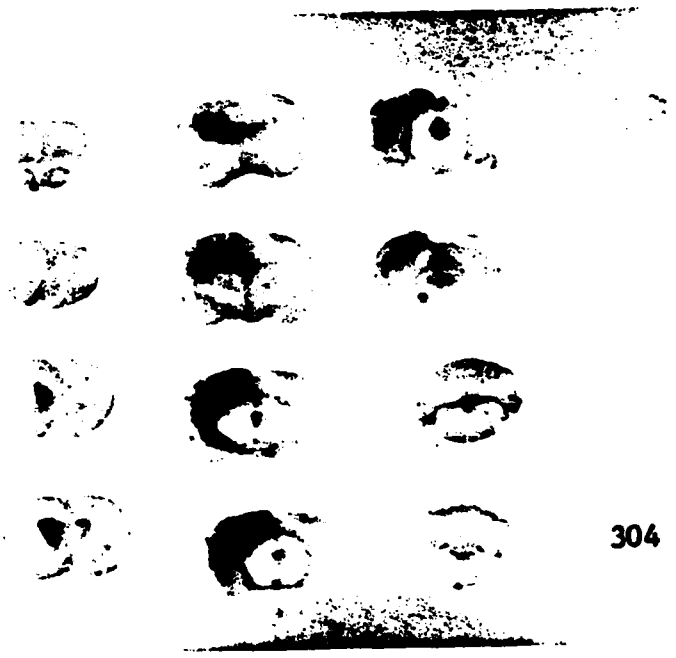

Figure 6. Brain sections (1 mm) from rat irradiated with 3 W/cm^2 of 2450-MHz microwave energy for 15 min.

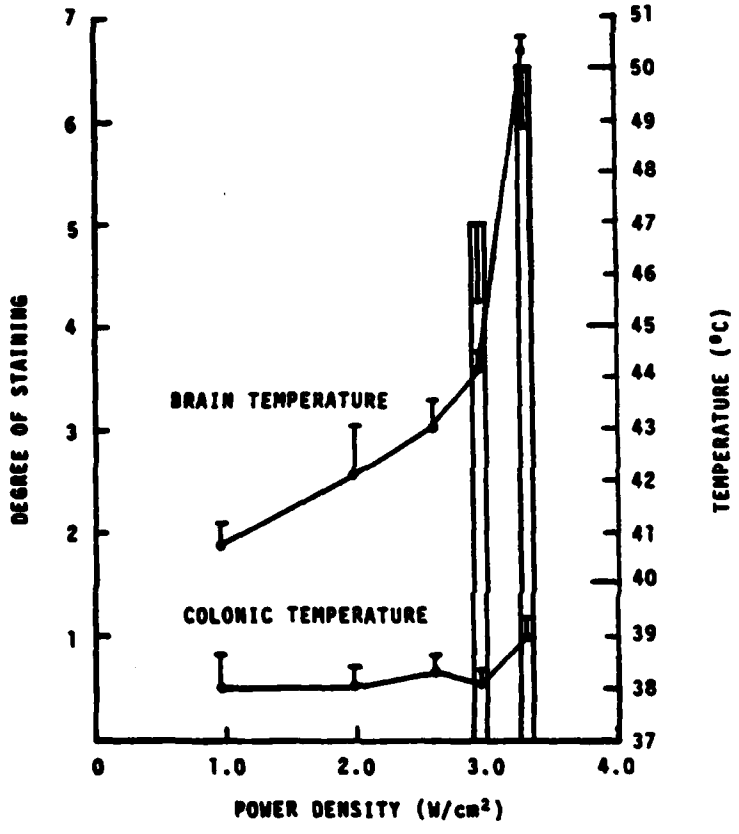

Figure 7. Power density dependence of microwave-induced blood-brain barrier permeation by Evans blue, and associated brain and colonic temperatures. Each point represents Mean ± SD of 3 or 4 animals.

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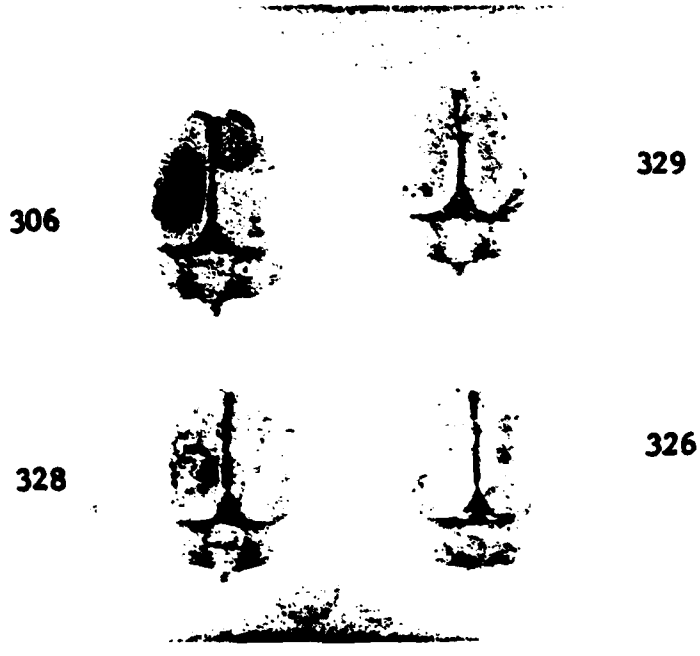

Figure 9. Rat brains illustrating reversibility of microwave-induced blood-brain barrier permeation. Irradiation parameters: 2450-MHz, 3 W/cm^2, 10 min. Protocols: 306, immediately following irradiation; 328, 5 min post-irradiation; 329, 10 min post-irradiation; 326, 30 min post-irradiation.

Figure 10. Brain sections (1 mm) from irradiated rat. Evans blue was injected immediately after irradiation.

Temperature measurements are condensed in Figure 7. The colonic temperature increased by about 1°C for incident power densities from 1 to 3 W/cm^2 (SARs of 80 to 240 mW/g in the cortex) and about 2°C when the incident power density was raised to 3.25 W/cm^2 at the end of 20 min of irradiation. The increase in cortical temperature was substantially higher and ranged from 4 to 13°C for SARs between 80 and 260 mW/g in the cortex. It is interesting to note that the mean cortical temperature did not exceed 43°C in animals that did not exhibit an increase in barrier permeability, whereas animals displaying increased permeation to Evan blue, had a cortical temperature greater than 43°C.

Typical cortical temperature-time profiles of rats whose head were irradiated with 1 and 3 W/cm^2 (SAR of 80 and 240 mW/g, respectively) are shown in Figures 12 and 13. In each case, the cortical temperature rapidly increased and reached 80 persent of its final value within 2-3 min. During the rest of the 20 min irradiation period, the temperatures rose more slowly as the thermoregulatory process attempted to cope with the microwave-induced heating. Note that the cortical temperature for the 1-W/cm^2 animal never exceeded 41°C whereas the 3-W/cm^2 animal had a cortical temperature that surpassed 43°C through most of the 20-min irradiation period.

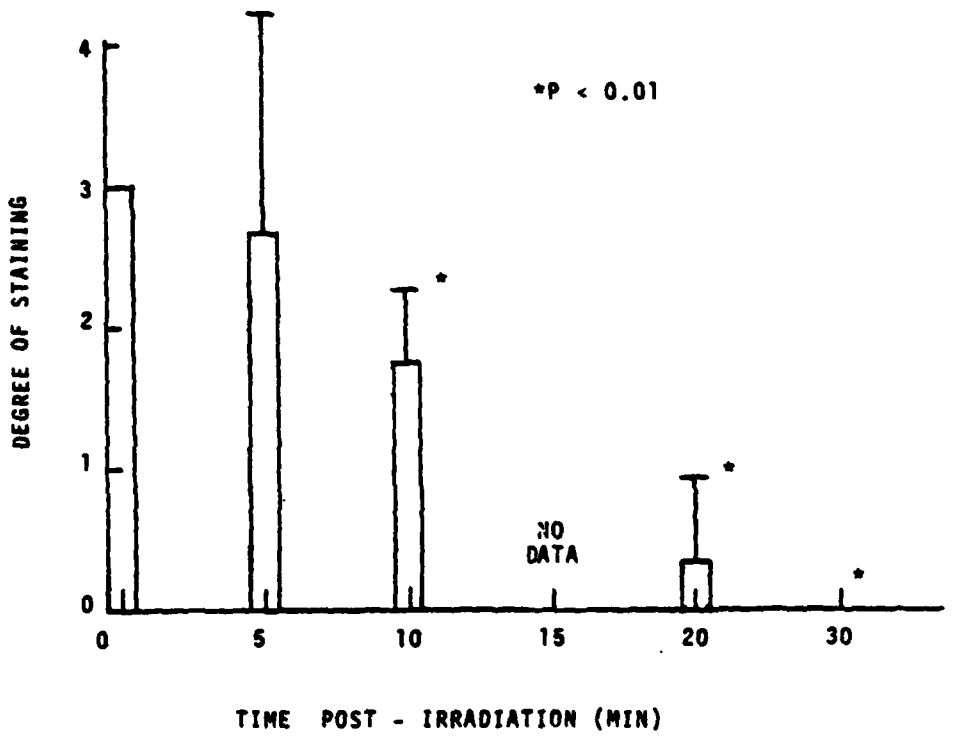

Figure 11. Reversibility of microwave-induced barrier permeation. Each point represents Mean ± SD of 3 or 4 animals.

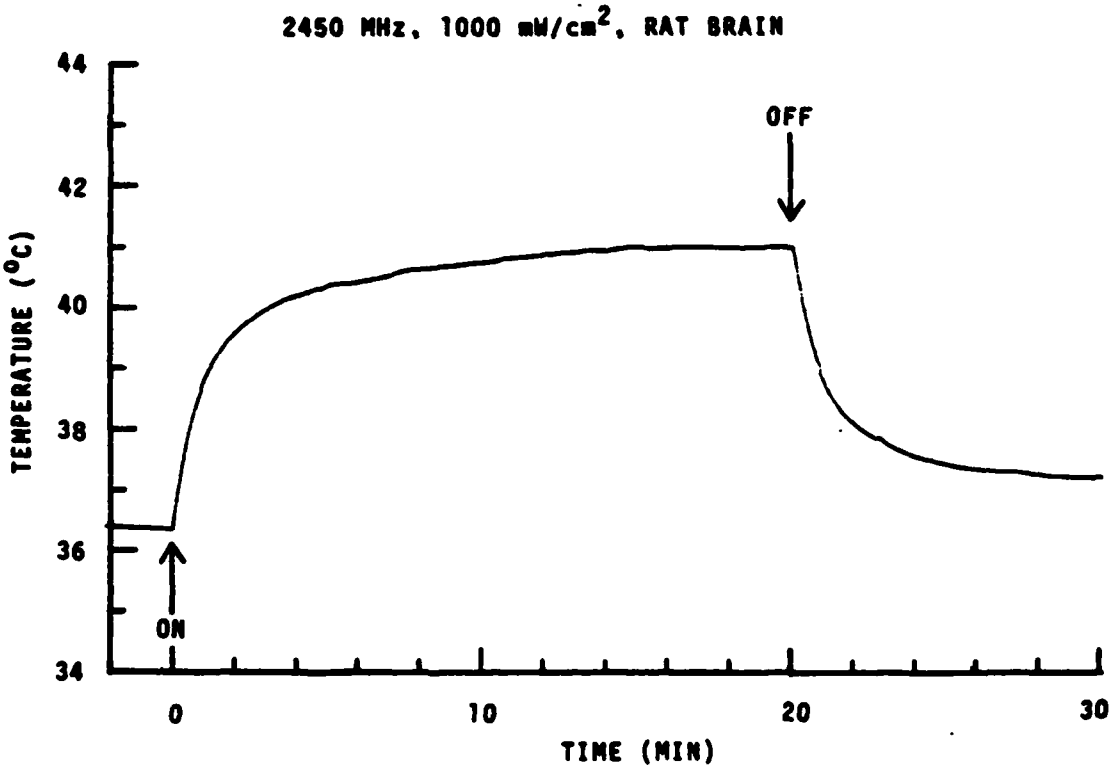

Figure 12. Brain temperature-time profile of a rat receiving radiation to the head of 1000 mW/cm^2 (80 mW/g) for 20 min.

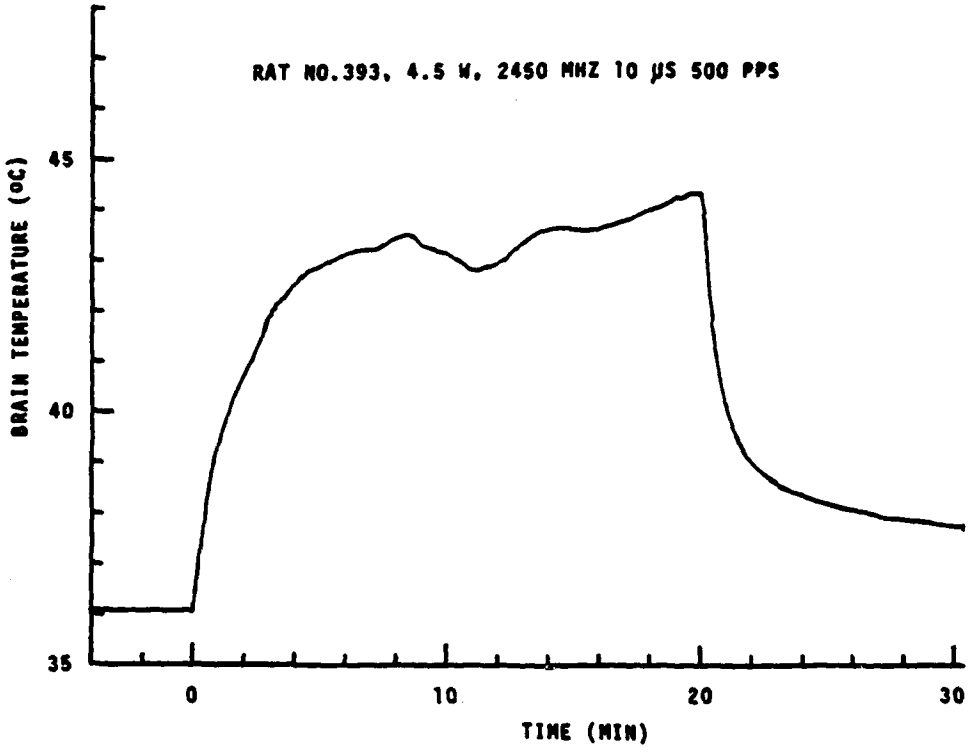

Figure 13. Brain temperature vs time of a rat undergoing a 20 min selective head irradiation at 3000 mW/cm^2 (240 mW/g).

DISCUSSION

The results of gross and microscopic examination of Evans blue dye transgression through the blood-brain barrier showed that intense microwave irradiation can alter the barrier's permeability. The temperature in brain regions under such conditions generally exceeded 43°C. These data imply that temperature in affected brain regions must exceed a critical value in order to effect microwave-induced alterations in blood-brain barrier permeation. This observation finds support in the work of Sutton, et al. (9, 10) which showed that the barrier to peroxidase could be disrupted by selective irradiation of the head for 15 min. This regimen raised the brain temperature of normothermic rats to 42°C.

Furthermore, these studies showed that the extent of Evans blue penetration into brain tissues and the degree of staining were diminished in animals allowed 20 min recovery before euthanasia. In addition, brains of rats euthanized 30 min after irradiation were free of Evan blue dye. This suggests a total reversibility of microwave-induced blood-brain barrier alterations.

Finally, microwave-induced changes in blood-brain barrier may have pathogenic as well as therapeutic implications. They may allow chemotherapeutic agents, antibiotics or neuroactive drugs which under normal conditions do not penetrate the blood-brain barrier, access to the brain to combat infections or other malignant processes. There is also, however, the possibility of promoting entrance of metabolites, humoral or other toxic agents into a immunologically priviledged brain. Thus, microwave-

induced blood-brain barrier permeability changes are significant from both safety considerations and medical applications.

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER BES-4 ✓	2. GOVT ACCESSION NO. AD-A091 047	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Mechanisms of Microwave-Induced Blood-Brain Barrier Alterations		5. TYPE OF REPORT & PERIOD COVERED Final Technical 4/1/78 - 8/3/80
		6. PERFORMING ORG. REPORT NUMBER BES-4
7. AUTHOR(s) James C. Lin, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) N00014-78-C-0353 NEW
9. PERFORMING ORGANIZATION NAME AND ADDRESS Wayne State University Detroit, MI 48202 ✓		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 201-330
11. CONTROLLING OFFICE NAME AND ADDRESS Office of Naval Research 800 North Quincy Street Arlington, Virginia 22217		12. REPORT DATE September, 1980 13. NUMBER OF PAGES 28
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Current Address: Bioengineering Program
P.O. Box 4348
University of Illinois (Circle Campus)
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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Microwaves, Rat, Blood-Brain Barrier, Evans blue, Power; Time and Reversibility.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

(See next page)

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ABSTRACT

This investigation was designed to study the mechanisms of interaction between microwaves and the blood-brain barrier and was aimed at correlating changes of blood-brain barrier permeability with the quantity and distribution of absorbed microwave energy inside the brain of adult Wistar rats under sodium pentobarbital anesthesia. Through use of thermographic methods and a direct-contact applicator at the animal's head, the pattern of absorbed microwave energy was determined. Indwelling cathethers were placed in the femoral vein. Evans blue in isotonic saline were used as a visual indicator of barrier permeation. Exposure to pulsed 2450-MHz radiation for 20 min at average power densities of 0.5 to 2600 mW/cm^2, which resulted in average specific absorption rates (SARs) of 0.04, to 200 mW/g in the brain, did not produce staining, except in regions that normally are highly permeable. When the incident power density was increased to 3000 mW/cm^2 (SAR of 240 mW/g), extravasation of Evans blue could be seen in the cortex, hippocampus and midbrain. The rectal temperature, as monitored by a copper-constantan thermocouple, showed a maximum increase of less than 1.0°C. The brain temperature recorded in a similar group of animals using a thick-film carbon thermistor exceeded 43°C. In one series of experiments, rats were irradiated at 3000 mW/cm^2 for 5, 10, 15 and 20 min. Immediately after irradiation all except the 5-min animals exhibited increased permeability in some regions of the brain. Brains of rats euthanized 30 min after irradiation were free of Evans blue, while those euthanized 10 and 20 min post-irradiation showed significant dye staining but with less intensity than those euthanized immediately after irradiation. These data suggest a total reversibility of microwave-induced blood-brain barrier changes within 30 min post-irradiation.