Title: Effects of Microwave Radiation on Humans

Subtitle: Monkeys Exposed to 1.25 GHz Pulsed Microwaves

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Report Date: March 13, 1992

Type of Report: Final Report

Prepared For: U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21702-5012

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1. GENERAL INTRODUCTION

This report is divided into 4 sections: general diagnostic evaluation; fluorophotometry; electroretinography; and histopathologic evaluation. At the end of the report, the results of all aspects of this study are summarized and discussed, and the findings are related to the exposure system. There is also a discussion of these findings in relation to recent observations on humans exposed to microwave.

All experimental animals were restrained without the use of anesthetics for the irradiation or sham irradiation portions of this study, which consisted of three sets of microwave exposures (three consecutive days of four hr irradiations/week, for a total of three weeks). One animal however, 6D, was initially irradiated over a three week period (2 days the 1st week, 2 days the 2nd week and 3 days the 3rd week) for 4 hours each day. The animal was allowed to rest for several weeks and then irradiated again (3 four hour exposures) prior to sacrifice.

Each animal was evaluated using a variety of in vivo diagnostic techniques prior to and following the final irradiation. These consisted of baseline and final electroretinography, fundus and cross polarization photography, corneal specular microscopy, and slit lamp observation. Aqueous humour fluorophotometry, consisting of three baseline measurements and measurements following each set of irradiations was also performed. Following the final in vivo diagnostic procedures, animals were euthanized and eyes enucleated for histopathological analysis. Three experimental monkeys and one control animal (sham exposed) were infused with horseradish peroxidase prior to euthanasia in order to examine ocular blood barriers.
2. CLINICAL DIAGNOSTIC EVALUATION

The monkeys in this study were subjected to a complete regimen of ophthalmic examinations and photographic documentation, pre and post irradiation. The ophthalmic examinations included slit lamp examination of the anterior chamber, lens, iris, cornea and corneal endothelial cells. The aqueous was examined with a slit lamp for cell and flare, and particulate matter. The entire retina was examined by indirect ophthalmoscopy.

Photographic documentation included photomicrographs of the corneal endothelial cells and retinal photographs of the entire posterior pole (8 stereo fields). The macula of each eye was also documented using cross polarization photography. In some instances where fluorophotometry was not possible due to equipment failure, iris angiography was performed to demonstrate possible leakage of dye from iris vessels into the anterior chamber.

A. Results of the ophthalmic examinations and photodocumentation:

MONKEY #13-E: In the pre and post-exposure ophthalmic examinations of the anterior chamber and posterior pole, the eyes appeared normal. The pre and post-exposure photos of the corneal endothelial cells are comparable and appear normal. Retinal photos, pre and post irradiation, likewise appear normal.

MONKEY #19-D: Ophthalmic examinations of the anterior chamber and posterior pole presented eyes that appeared clean and normal. The corneal photomicrograph presented a mosaic pattern that was somewhat granular. However, this granularity was present pre and post irradiation and could be interpreted as normal for this animal. Retinal photodocumentation taken pre and post irradiation also appear normal.

MONKEY #4-E: Pre and post-exposure ophthalmic examinations of anterior segment and retina appear normal. Photos of corneal endothelium pre and post irradiation demonstrated no change. The retinal photos also appeared normal.

MONKEY #27-E: Pre and post ophthalmic exam determined that the anterior chamber and posterior pole appeared normal. Corneal endothelium appeared somewhat granular but normal. The retinal photos also appeared normal.
MONKEY #6-D: This monkey underwent the routine ophthalmic and photographic documentation. However, baseline fluorescein angiograms of the retina and iris were also included. It was determined that baseline and post irradiation examinations and photos of the retina appeared normal. The corneal endothelium was monitored 5 times. The baseline OD did present some scattered spots, OS appeared normal throughout. The endothelium appeared papery but normal.

In summary, no clinical level changes (gross changes) were observed. In other words, all monkeys were normal by these clinical diagnostic procedures after the irradiation protocol.

3. FLUOROPHOTOMETRIC ANALYSIS OF THE BLOOD-AQUEOUS BARRIER IN EXPERIMENTAL MONKEYS

Our previous studies which examined the effects of microwave radiation on the eye have shown that experimental monkeys exposed to low level pulsed microwaves at 2.45 GHz demonstrate increased permeability of the blood-aqueous barrier to sodium fluorescein by angiography and horseradish peroxidase. In this study, we attempted to quantify blood-aqueous permeability changes with fluorophotometry. The fluorophotometer is a highly sensitive computerized system which accurately measures the fluorescein concentration in the aqueous or vitreous humour after intravenous injection. Fluorescein concentrations in the anterior chamber represent contributions of dye from the blood-aqueous barrier (ciliary body and iris vasculatures and secretion by the ciliary processes). Presented in this section of the report are fluorophotometric data from four monkeys exposed to pulsed microwaves at 1.25 GHz and two sham exposed control monkeys. Only six of the 7 rhesus monkeys were used in this aspect of the study due to equipment failure.

Aqueous humour fluorophotometry was performed using a Coherent Radiation Fluorotron Master fluorophotometer. Aqueous humour dye concentrations were measured prior to microwave exposures (baselines), and immediately following each set of microwave exposures. For performing fluorophotometric measurements, the animal was immobilized with an intramuscular injection of 0.3 ml Ketamine, intubated and placed on
Halothane/O₂ (2.5% Halothane, 97.5% O₂, 0.5 L/min). A preinjection scan was made for each eye prior to injection to determine baseline autofluorescence. An intravenous injection of 0.3 ml of 10% sodium fluorescein was made in the saphenous vein and scans were performed at 15 min intervals for 90 min. In each case, right eyes were scanned first, followed within two minutes by the left eye. Scans of the lens and anterior chamber were analyzed using 8 point averaging. Lens fluorescence did not change significantly during the scanning period.

A. RESULTS

In all cases, a minimum of two baseline determinations per animal were obtained prior to microwave exposures. In some cases as many as three baselines were obtained. Also, equipment failures resulted in the inability to obtain several sets of data. Statistical analysis has not been performed on any of the data presented within. Data collected over the course of this study is summarized in Fig. 1. The values below and in Fig. 1 are averages of all baseline readings or averages of all values after microwave exposure.

Microwave Exposed:

19D OD. Compared with baseline data, there appeared to be a trend of decreasing blood-aqueous barrier permeability after early microwave exposure. However, the average value from all post-exposure readings was comparable to the baseline value average, as in the shams.
Baseline average @ 90 min. = 172.3 ng/ml
Microwave exposed average @ 90 min. = 177.6 ng/ml

19D OS. Data from this eye were similar to the fellow eye.
Baseline average @ 90 min. = 156.1 ng/ml
Microwave exposed average @ 90 min. = 156.4 ng/ml

13E OD. Data from this animal also suggested a slight decrease in blood-aqueous barrier permeability following microwave exposure.
Baseline average @ 60 min.* = 215.3 ng/ml
Microwave exposed average @ 60 min.* = 171.2 ng/ml
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13E OS. Data from this eye was similar to OD.
Baseline average @ 60 min.* = 218.8 ng/ml.
Microwave exposed average @ 60 min.* = 153.7 ng/ml
* Note - Sixty minute scans are presented due to lack of 75 and 90 min. data following 1st set of microwave exposures.

4E OD. Following the 1st set of microwave exposures there was a significant increase in blood-aqueous barrier permeability in this animal which decreased following the next two sets to near baseline levels.
Baseline average @ 90 min. = 226.4 ng/ml
Microwave exposed average @ 90 min. = 337.4 ng/ml

4E OS. Same as OD, an increase in permeability.
Baseline average @ 90 min. = 188.8 ng/ml
Microwave exposed average @ 90 min. = 278.0 ng/ml

27E OD. Blood-aqueous barrier permeability increased significantly following the 1st and 2nd sets of exposures. Following the third set, however, values decreased but were still above baseline levels.
Baseline average @ 90 min. = 162.1 ng/ml
Microwave exposed average @ 90 min. = 243.2 ng/ml

27E OS. Same as OD.
Baseline average @ 90 min. = 151.5 ng/ml
Microwave exposed average @ 90 min. = 259.9 ng/ml

Sham Exposed

21E OD. Baseline average @ 90 min. = 161.5 ng/ml
Sham exposed average @ 90 min. = 161.0 ng/ml

21E OS. Baseline average @ 90 min = 148.9 ng/ml
Sham exposed average @ 90 min. = 158.4 ng/ml
22E OD. Baseline average @ 90 min = 90.7 ng/ml  
Sham exposed average @ 90 min. = 105.6 ng/ml

22E OS. Baseline average @ 90 min = 99.9 ng/ml  
Sham exposed average @ 90 min. = 112.5 ng/ml

B. CONCLUSIONS

Shown below are plots for each animal of averaged data for 90 minute scans made before microwave exposure (baseline measurements) and plots of averaged data for 90 minute scans following microwave exposures (post exposure) over the course of this study. Right eyes are compared in Figure 1A while left eyes are compared in Figure 1B. Of the four experimental animals used in this study, only 4E and 27E demonstrated increased permeability of the blood-aqueous barrier following microwave exposure. Of the two remaining animals, 19D showed no change while 13E demonstrated decreased permeability. The sham controls showed little or no changes following sham exposures.

Based on these data, the order of most affected blood-aqueous barrier to least affected was 4E, followed by 27E, 19D and 13E.
FIGURE 1: AVERAGE BASELINE FLUOROPHOTOMETRIC VALUES VERSUS POST-MICROWAVE VALUES FOR (A) RIGHT EYES AND (B) LEFT EYES. POSTMICROWAVE VALUES ARE THE AVERAGE OF ALL POST-MICROWAVE DETERMINATIONS.
4. ELECTRORETINOGRAPHIC ANALYSIS

Electroretinography is used to detect functional changes (electrical) in retinal response to light. Two ERG parameters were evaluated in the monkeys used in this study: 1. single flash response which is a response of mostly rods; and 2. flicker response which measures cones because rods are not sensitive enough to respond to these quick flashes of light. Each animal was evaluated by both parameters before exposure (baseline) and then after the last exposure.

A. RESULTS

The results were bilateral and therefore the response of each eye is not given.

27E- Microwave-exposed animal with normal baseline responses and who showed very little change in amplitude of response from baseline.

19D- Amplitude of rod response was 58% of baseline response.
    Amplitude of cone response was 27% of baseline response.

6D- Amplitude of rod response was 44% of baseline response.
    Amplitude of cone response was 11% of baseline response.

13E- Most affected cone ERG response. Amplitude of rod response was 48% of baseline response. Amplitude of cone response was 0% of baseline response.

Responses of both sham-exposed animals were unchanged from baseline values. The results are summarized in Figure 2. The actual scans are shown in Figures 3-7.
B. Response Variability

Ten ERGs were recorded on ten separate days during a two week period in order to determine intrasubject variability for ERG amplitudes. The ERG variability was similar to that observed in humans. For scotopic b-wave amplitudes, one standard deviation measured 45.7 μV OD and 42.6 μV OS, which represented 16% and 15% of the mean value, respectively. (The comparable value for humans is 18%). For photopic b-wave amplitudes, 1 standard deviation measured 19.2 μV, or 13% of the mean. These data allow us to more precisely determine the significance of an amplitude reduction in an experimental animal.

FIGURE 2: SUMMARY OF ERG RESPONSES.
FIGURE 3: ERG RESPONSES FROM MONKEY 27E.

A. 27 E Primarily Rod Response

B. 27 E Cone Response

5 ms per Division
FIGURE 4: ERG RESPONSES FROM MONKEY 4E.

A. Baseline Rod Exposure

B. Cone Exposure

4E Baseline Rod

5 ms per Division

4E Baseline Cone

5 ms per Division
FIGURE 5: ERG RESPONSES FROM MONKEY #19D.

A. 

B. 

19 D — Primarily Rod Response

19 D — Cone Response
FIGURE 6: ERG RESPONSES FROM MONKEY #6D.

A. 6 D Primarily Rod Response

B. 6 D Cone Response
FIGURE 7: ERG RESPONSES FROM MONKEY #13 E.

A.

B.
5. HISTOLOGICAL EVALUATION OF RETINAL TISSUES

All tissue was processed as described previously and embedded in glycol methacrylate. Sections were taken through the macular and foveal regions of each eye. Evaluation of retinal sections was performed by two independent observers (one in a masked fashion). Final analysis was made collectively by both observers in a masked fashion.

A. RESULTS

The most striking and substantive morphological change observed in retinas of microwave-exposed animals was the degenerative appearance of cone nuclei in the macula. The degree of degeneration was comparable in both eyes of a given monkey. The number of affected cones varied between animals. The cytological characteristics of this degenerative change were loss of basophilia of nuclear material and clumping of chromatin. In most cases the nuclear envelope appeared intact, however, the inner and outer segments of these cones were often retracted and vacuolated. In some cones, karyorrhexis (fragmentation of nucleus) had occurred. These degenerative changes in photoreceptor cell nuclei will be called collectively karyolysis in this report. A representative section from one eye of each animal is presented in Figures 8 and 9.

MICROWAVE-EXPOSED ANIMALS

6D, OD. Various degrees of cone nuclei karyolysis were evident within the foveal region and scattered throughout the macula. Occasional cones in nasal retina were also affected. No morphological changes were observed in rods or retinal pigment epithelium (RPE).

6D, OS. This retina had the same degree of damage as 6D, OD.

19D, OD. Karyolysis of cone nuclei was observed in central fovea and in nasal macula. No morphological changes were seen in rods or RPE.

19D, OS. Changes were comparable to 19D, OD.
13E, OD. Extensive karyolysis of cone nuclei was observed throughout the retina. The greatest number of karyolytic cone nuclei was in fovea. Karyolytic rod nuclei were also observed throughout retina but the percentage of the population affected was less than in the cone population. RPE cells had pyknotic nuclei and scant cytoplasm. Some vessels in both retina and choroid appeared partially occluded. Yellow vesicular material was observed in some vascular lumens.

13E, OS. Changes to rods, cones, and RPE cells was as extensive as in the fellow eye, 13E, OD.

4E, OD. No apparent changes were observed.

4E, OS. There were no apparent changes in retinal tissue.

27E, OD. Occasional cone nuclei karyolysis was observed in nasal retina and in fovea and macula. There were no apparent changes in rods.

27E, OS. There were more affected cones in this eye than in 27E, OD, but the overall change was quite moderate.

SHAM EXPOSED:

22E, OD. No apparent changes were observed.

22E, OS. No apparent changes were observed.

21E, OD. Occasional scattered karyolytic cone nuclei in macula.

21E, OS. There were no apparent changes in retinal tissue.

B. CONCLUSIONS

The experimental monkeys used in this study demonstrated varying degrees of photoreceptor changes which were primarily confined to cones in the macula.
They were ranked in this order from most severe to least based on relative number of karyolytic cone nuclei, pyknotic rod nuclei, and change in appearance of RPE: 13E, 6D, 19D, 27E, and 4E. In cases with extensive cone cell degeneration, the RPE cells were also affected. Of particular interest was the fact that, in general, eyes which showed an effect had very few degenerative cones outside the macular region. The exception to this was 13E which demonstrated photoreceptor cell degeneration throughout the retina. Moreover, within the macula of the moderately affected eyes, the distributional arrangement of karyolytic cone nuclei appeared in a pattern such that every fourth to fifth cone nucleus in the outermost portion of the outer nuclear layer appeared degenerative. This was best illustrated in moderately affected animals such as 27E. Detailed analysis of sections taken from different areas of retina revealed degenerative cones only within the parafoveal region, an area which has been shown to contain the highest number of blue-sensitive cones. Blue cone function unfortunately can not be measured with our ERG equipment to date. It is tempting to speculate that a specific population or subpopulation of cones were more susceptible to microwaves.
FIGURE 8: TWO MICRON GLYCOL METHACRYLATE SECTIONS THROUGH THE Fovea.

A. 22E, OD
Sham.
Arrows show the appearance of normal cones.

B. 4E, OD
No change.

C. 27E, OD
Scattered cone karyolysis (arrows).
FIGURE 9: TWO MICRON GLYCOL METHACRYLATE SECTIONS.

A. 21E, OS
Sham.

B. 19D, OS
Scattered cone karyolysis (arrows).

C. 6D, OS
Majority of cones karyolytic.

D. 13E, OS
All cones karyolytic.
6. OVERALL SUMMARY AND GENERAL CONCLUSIONS

Although no clinical level (gross) changes were observed (fundus and cross polarization photography, corneal specular microscopy, and slit lamp observation), we did observe changes after microwave exposure with fluorophotometry, ERG, and histopathology. Experimental animals are listed in Figure 10 from those whose ERG was most affected (13 E, top) to one that had no change in ERG (27E, bottom). The histologic examination was in agreement with ERG in that 13E was most affected, then 6D, and 19D. However, contrary to ERG data we saw no morphologic changes in 4E and some changes in 27E. If damage in 27E was in blue cones, our current ERG protocol would not have been capable of detecting the damage. Of particular interest is the fact that the animals with the most severe changes in the anterior segment (increase in permeability of the blood aqueous barrier) were the animals that showed the least retinal histopathologic changes and, conversely, the animals with the most histopathologic damage in retina had the least affected anterior segment. In a preliminary histological evaluation of some of the anterior segments from the study, the two animals with increased blood-aqueous barrier permeability also had degenerative changes in corneal endothelium. The depth of the affected tissue in the eye could be related to the concentration of melanin in the iris and ciliary body, but all of these animals were Rhesus monkeys and had comparable pigmentation. The experimental animals fell, therefore, into two groups: (1.) animals with functional and structural changes in retina and no change in blood aqueous barrier (13E, 19D, and 6D); and (2.) blood aqueous barrier changes and slight or no change in retinal function or morphology (4E and 27E). In the middle of this study the exposure system was changed. It is unclear if that change was affected the microwave field. Group 1 was irradiated first chronologically, and group 2 were irradiated last after the exposure system was changed. The suggestion is that after the change in system, energy was deposited in anterior chamber and not at the retina.

The effect of microwave on ERG response has never been investigated before. Our results suggest that this may be a simple non-invasive test for microwave exposure. How much function loss is permanent is yet to be determined. Recently two patients accidentally exposed to microwave have been evaluated at Wilmer and they had symptoms of ERG cone functional loss and extreme photophobia. One patient had no recovery of function. The humans like our monkeys had no gross clinical level changes. Based on our knowledge of photoreceptor renewal
mechanisms we might speculate that probably all but the loss of photoreceptor nuclei is reversible to some extent. The only animal that meets these criterion of photoreceptor death was 13E.

The extent of microwave-induced photoreceptor cell changes in the macular region of experimental animals, could perhaps be best demonstrated by morphometric analysis of tissue sections. We feel it is in the best interest of publishing this data if the degree of photoreceptor cell degeneration could be quantified by morphometry. This can now be accomplished with an image analysis system available in G. Lutty's lab, but still would require a considerable amount of someone's time. It would be interesting to compare the morphometric results to changes in blue cone function. We will be able to measure blue cone function in the near future with equipment that M. Johnson is creating.

FIGURE 10:

SUMMARY OF 1.25 GHz MICROWAVE ARMY STUDY

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>FLUOROPHOTOMETRY</th>
<th>HISTOLOGY</th>
<th>ERG (% of NORMAL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(affected cells)</td>
<td>ROD</td>
</tr>
<tr>
<td>13E</td>
<td>Slight decrease permeability</td>
<td>Cones and rods</td>
<td>48%</td>
</tr>
<tr>
<td>6D</td>
<td>N/D</td>
<td>Cones</td>
<td>44%</td>
</tr>
<tr>
<td>19D</td>
<td>No change</td>
<td>Cones</td>
<td>58%</td>
</tr>
<tr>
<td>4E</td>
<td>Increase permeability</td>
<td>No change</td>
<td>72% (NS)</td>
</tr>
<tr>
<td>27E</td>
<td>Increase permeability</td>
<td>Few cones</td>
<td>119% (NS)</td>
</tr>
</tbody>
</table>

(NS= not significant)
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