

PHYSIOLOGICAL AND BEHAVIORAL CHANGES IN RATS EXPOSED
PRENATALLY TO 2450-MHz 500 μ W/cm² MICROWAVES

Eight pregnant female rats were exposed 20 hr/day during the first 19 days of gestation to 500 μ W/cm², 2450-MHz microwave fields without apparent adverse effect upon their general health although their percent increase in bodyweight during this period as compared to sham-exposed controls was significantly lower. At parturition there were no offspring with discernable deformities, nor were total births or mean birth weights significantly different. However, during the first week of life there was a sevenfold increase in mortality among the prenatally exposed offspring and significantly lower average bodyweights. In addition, these pups were significantly retarded in their rate of eye openings during the second week of life. First week bodyweight differences were no longer evident by the second week nor could the offspring be differentiated on bodyweight through puberty. However, in apparent coincidence with puberty, the prenatally exposed female pups experienced an accelerated growth pattern. When tested as adults these females demonstrated a significant increase in avoidance responding during acquisition of a conditioned avoidance response and an significant response to a series of colonic temperature measurements. A previous study of prenatal exposures to 918-MHz reported some similiar and some disimiliar effects from those reported here - suggesting involvement of both unique and common mechanisms. Discussion includes the importance of frequency vs SAR in the interpretation of these effects.

SUMMARY

This study represents our continuing efforts to investigate chronic low-level effects of microwave irradiation upon biological systems. It replicates in essence the protocol used in a previously reported study on the effects on rats exposed in-utero to 918-MHz microwaves at a field density of 5 mW/cm² (Johnson, Mizumori, Myers, Guy and Lovely, 1978).

SUBJECTS

Thirty Wistar-derived female rats obtained from the colony maintained by the vivarium of the Psychology Department at the University of Washington served as exposed, sham-exposed and non-handled controls in this study. They were introduced into our lab at approximately 85 days of age, weighing 200 to 300g. Ordered on bodyweight they were quasi-randomly assigned eight per group to form treatment groups of approximately equal average bodyweight and individually housed in hanging metal cages with wire mesh floors. Fluorescent room lighting was maintained on a 12 hour light/dark cycle (lights on 7:00 a.m. to 7:00 p.m.). Room temperature and relative humidity were monitored daily at 20 ± 1 C and 50 ± 5%. Throughout experimentation all animals were maintained with ad libitum access to Purina Lab Chow (#5001) and tap water.

APPARATUS

The exposure system consisted of 16 cylindrical wire-mesh waveguides capable of delivering 2450-MHz circularly polarized guided waves. This system allows for easy quantification of field parameters and has been described in detail elsewhere (Guy, 1977). The animals resided in Lucite living chambers inside the waveguide, with access to food and water. The nesting boxes used during the last few days of gestation and through weaning were wood-chip filled opaque vinyl tubs measuring 25 x 32 x 15 cm with hardware cloth lids.

Adult conditioning assessments were conducted in the two homogeneous shuttlebox apparatuses used in the earlier study (Johnson, et al 1977). Each shuttlebox, 67 by 18 by 18 cm high was constructed with a center-pivoting grid floor of 1-cm-diameter stainless steel bars spaced on 2.5 cm centers. Movement of a rat 8 cm beyond the center line on either side offsets the floor in that direction. Through the use of microswitches, concealed photocells and opposing red light sources a microcomputer system monitors a rat's shuttle behavior within the box and determined response classification, latencies, running speeds etc.. The system also controls trial initiation through presentation of a 2000-Hz tone (CS) and delivery of scrambled 1-mA 350-V a-c discontinuous (0.5 sec on / 0.5 sec off) electric shock (US) to the grid floor.

DOSIMETRY

Four additional pregnant female rats with an average bodyweight of 336 ± 16gm were used to make thermographic determinations of SAR (this compares well with the average bodyweight of the exposed females during gestation of 349 ± 50gm). When placed in a waveguide

with 1W input, these females absorbed an average of $81 \pm 3\%$ of input power giving an average SAR of $2.3 \pm .15$ per W input. Under the actual conditions employed in this study, each of the eight exposure waveguides were delivered an average of 181 ± 15 mW to establish an averaged cross-sectional field intensity of $500 \mu\text{W}/\text{cm}^2$. This gives an average SAR of $.416 \text{ W}/\text{kg}$ for pregnant female rats under these exposure conditions.

PROCEDURE

After five days of home cage adaptation, the 20 rats to serve as exposed or sham-exposed subjects began a 2-week adaptation to waveguides. This was accomplished through the use of additional waveguides normally used in 918-MHz studies. Since no radiation was presented during this period all waveguides can be considered identical. These animals were placed in their respective waveguides daily at noon and remained there until 8:00 a.m. the following morning at which time they were removed to home cages to allow for maintenance of the waveguide living chamber. Body weight was measured daily upon removal and food and water consumption during the previous 20 hr period in the waveguide calculated. The 10 remaining females remained in home cages throughout this week and eventually served as caged controls.

After this adaptation phase, four females from each of the three treatment groups were placed individually with one of 12 experienced males in his home cage. Tray papers under the males were checked daily at 8:00 a.m. for evidence of sperm plugs. When conception was judged to have occurred the dame followed its respective treatment protocol until the 20th day of gestation. For an exposed or sham-exposed female this consisted of 20 hr daily waveguide habitation as during adaptation with the associated handling, weighing etc.. Caged controls were returned to home cages to remain undisturbed for 19 days. On day 20 of gestation a female was removed from her home cage at 9:00 a.m. and placed in a nesting box.

As each female was removed from a males home cage she was replaced by another female representing a different treatment group. This procedure was repeated until 8 females were impregnated for each condition.

Nesting boxes were examined twice daily at approximately 8:00 a.m. and 5:00 p.m. for deliveries. After a delivery had occurred the pups in that litter were temporarily removed, weighed, counted and inspected for signs of nursing. At four days of age, the pups were again removed for sexing and each litter was culled to four males and four females. Weekly, beginning on day 7 through day 28 the pups were removed briefly for weighing. From day 10 to day 17 each pup was examined twice daily for eye openings. After weighing on day 28 the pups were removed from the mother and some individually housed and some group housed. After all litters had been weaned, six groups of 15 rats each were individually housed (male and female; exposed, sham and control groups) and unequal numbers (6 to 8 per group) were group housed. The individually housed animals were weighed weekly through adulthood.

At 90 days of age, eight representatives from each of the six individually housed treatment groups began shuttlebox avoidance conditioning. Two animals were tested daily, randomly chosen from one

of the treatment groups. Testing of the females began only after all male testing was complete. A test session consisted of 200 trials with a randomly varying inter-trial interval (ITI) averaging 90 ± 45 sec. During the first 100 trials (acquisition phase) a trial consisted of the presentation of the CS followed 10 secs later by delivery of the US. In the absence of any response both the tone and shock terminated automatically 30 secs into the trial. If however the rat traversed the runway within the initial 10 secs this terminated the tone and obviated the delivery of shock. This response was scored an avoidance. After presentation of shock a traverse terminated both tone and shock and counted as an escape response. The second 100 trials were identical except that shock was never presented with the tone terminating after 30 secs in the absence of responding.

Also at 90 days of age, the remaining seven animals from each of the six individually housed groups began adaptation to the handling procedure used in determination of the circadian rhythm of colonic temperature. After this adaptation these animals were each probed 5 times over a 24-hour period at six hour intervals beginning at noon. A thermister probe was used and colonic temperatures read from a digital thermometer to within $\pm .10$ C.

RESULTS and DISCUSSION

During gestation the exposed dames increased bodyweight an average of 9gm less than the sham-exposed dames which on a percentage basis is statistically significant ($p < .01$). Likewise, in the 918-MHz study the exposed dames gained 7gm less than sham-exposed dames. This similarity is somewhat surprising in that whole-body SAR for these dames was 2.5 W/kg - a six fold increase in SAR as reported here for 2450-MHz.

At 918-MHz there were no differences in total births between conditions nor any observable abnormalities. However, the prenatally exposed offspring were significantly heavier at birth and demonstrated an accelerated rate of eye opening. In the present study again there were no differences in total births nor observable abnormalities. In contrast, there was during the first week of life an increased mortality among prenatally exposed offspring (7) versus (1) for each of the sham and control groups. In addition, all exposed offspring demonstrated a significantly ($p < .05$) lower bodyweight at the end of the first week and a significant ($p < .015$) retardation of initial eye opening.

Although bodyweight did not differentiate groups after the first week, beginning shortly after weaning the prenatally exposed females rapidly gained weight and then maintained a 10-20 gm lead on the sham and control female offspring throughout adulthood. When tested as adults in the shuttlebox these females demonstrated both a significantly greater total number of avoidances during acquisition and a lower trials-to-criterion score. In addition, whereas normal rat colonic temperature increases at night and returns to a lower level in the morning, the prenatally exposed females temperature rose steady at each successive measurement and remained significantly higher ($p < .01$) as the sham and control values returned to normal morning baseline.

The normal circadian pattern was observed in all subjects at

918-MHz and all male subjects at 2450-MHz. In final contrast to the present study where only the exposed females showed any shuttlebox effects, at 918-MHz all exposed offspring demonstrated a significantly lower number of total avoidances.

A possible hormonal basis for the adult effects that seem specific to the prenatally exposed females, including the unusual growth, apparent evoked-thermal-response (ETR) to the colonic probing and the shuttlebox facilitation might involve a prenatal microwave interaction with tissue later effecting estrogen metabolism.

REFERENCES

Johnson, R.B., Sheri Mizumori, D.E. Myers, A.W. Guy and R.H. Lovely. "Effects of Preand Post-Natal Exposures to 918-MHz Microwave Radiation On Development and Behavior in Rats." Abstracts of Scientific Papers, 1977 URSI International Symposium on the Biological Effects of Electromagnetic Waves. Oct. 30 - Nov. 4, Arlie, Virginia

Guy, A.W., and Jack Wallace. "Circularly Polarized 2450-MHz Waveguide System for Chronic Exposure of Rodents." Abstracts of Scientific Papers, 1977 URSI International Symposium on the Biological Effects of Electromagnetic Waves. Oct. 30 - Nov. 4, Arlie, Virginia