ELECTROMAGNETIC RADIATION EFFECTS ON THE BLOOD-BRAIN BARRIER SYSTEM OF RATS

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ABSTRACT

Rats were exposed to 1.3 GHz microwave energy to assess the uptake of various, relatively nondiffusible neutral polar substances in certain areas of the brain. A quantitative, radioactive isotope method, which uses the highly diffusible substance ^{3}HOH as an internal standard, was employed to measure the loss of ^{14}C-labeled test substances to brain tissue. A single, 20 minute exposure, to either pulsed or continuous wave (CW) microwave energy induced an increase in the uptake of D-mannitol at average power densities of less then 3.0 mW/cm^2. The permeability change was the greatest in the medulla, followed, in decreasing order, by the cerebellum and hypothalamus, with small or negligible changes in the hippocampus and cortex. Increases in permeability were observed for mannitol and inulin but not for dextran. Increased permeability was observed both immediately and 4 hours after exposure, but not 24 hours after exposure. Differences in the level of uptake occurred between CW energy and pulsed energy of the same average power density. Microwave radiations of the same average power but different pulse characteristics also produced different levels of mannitol uptake. Our findings suggest that microwave energy induces a temporary change in the permeability for small inert polar molecules in the blood-brain barrier system of rats.

INTRODUCTION

In the last few years several central nervous system (CNS) alterations have been shown to occur as a result of low power, nonionizing electromagnetic radiation (1-5). These findings are significant in the study of the interaction of electromagnetic energy with the CNS both as to safety concerns and as a new research tool in the study of brain function. Amplitude modulated fields (147 MHz, 1mW/cm^2) have been shown by Bawin and coworkers to strongly influence spontaneous and conditioned EEG patterns in the cat (6). Albert has observed neuronal swelling, vacuolation, and chromatolysis in the hypothalamic and subthalamic brain regions of Chinese hamsters exposed to a 10 mW/cm^2, CW microwave field (7). Reduced calcium efflux with oscillating extremely low frequency fields and increased calcium efflux with modulated very high frequency fields have been demonstrated

in isolated chick and cat brain (8,9). These efflux changes appeared to display inverted U shaped functions for modulation frequency and for amplitude of the incident wave. Such studies indicate that electromagnetic energy is causing changes in the CNS and the investigations which utilize quantitative techniques are very fruitful in uncovering possible mechanisms of interaction and their dependence on microwave parameters.

The blood-brain barrier (BBB) system has been used to investigate effects on the CNS of various types of physiological activity and stress, such as cold, heat, photic stimulation, pressure, ionizing radiation, seizures, drugs, sensory input (10-13). Bondy and Purdy have shown an increased penetrance of tyrosine into brain areas receiving reduced sensory input (14). Sabbot and Costin have shown increased uptake of ^{45}Ca in brain tissue as a result of cold stress conditions (15). Concussions have been studied experimentally by the creation of pressure pulses induced by the sudden introduction of a small volume of fluid extradurally through a parietal trephine hole (16). Low magnitude pressure pulses gave abnormal penetration of protein tracer within the walls of the blood vessels. Ionizing radiation, edema, anoxia, hypertension, drug induced convulsions, embolisms, osmotic imbalances, etc. have all been shown to cause BBB changes and increased permeability of substances to the brain. Recently, Frey has reported increases in the brain tissue permeability of rats to intravenously injected flourescein dye when exposed to low power, pulsed or CW microwave energy at 1.2 GHz (17).

In the present studies a quantitative radioactive isotope technique was used to corroborate and extend the work of Frey on microwave induced BBB permeability changes in brain. The technique, developed by Oldendorf, permits quantitative measurement of the relative amount of test substance entering a particular region of the brain in reference to a highly diffusible substance such as tritiated water (18,19). This method allows measurement of the penetration of a variety of test substances as a function of any of several variables such as microwave characteristics, brain region, time after exposure, molecular weight of test substance, etc. While this techniques does not allow the detailed localization of tracers and display of the BBB alteration site that staining and observation with an electron microscope might, it does permit limited mapping of tracer penetration. This technique also lends itself well to simple statistical interpretation as to whether a certain insult produces a BBB permeability change, even if the change is only a small one.

The present experiments explore the magnitude of change in brain tissue permeability to neutral polar substances of different molecular weights as a function of recovery time, brain region, and different combinations of microwave exposure parameters. Rats served as subjects and they were exposed in all tests to CW or pulsed microwaves at a frequency of 1.3 GHz.

MATERIALS AND METHODS

Male Wistar rats from the Walter Reed colony served as subjects in all experiments (20). When an individual rat had grown to a body weight of 230-270 g, it was scheduled for a single sham or microwave exposure that day between 0900 and 1500 hr. Day of the week, time of day, and body weight were balanced among test groups in each experiment. One or more control groups were included in each test.

A microwave anechoic chamber maintained at 70 ± 1°F was used for exposures. This chamber was approximately 5m wide by 5m high by 10m long. The inside surfaces were covered with wedge absorber (EC, WG-4) and areas of possible specular reflection were covered with pyramidally shaped absorber (EC, VHP-45). The resulting performance is such that reflected energy is approximately 40dB below the direct rays.

All exposures were at 1.3 GHz (wavelength of 23 cm). For CW exposures the transmitter consisted of a sweep frequency generator (Hewlett-Packard 8690B) and an amplifier (Alfred 5020) coupled to a standard gain horn (Narda Model 646). Only the horn was mounted in the anechoic chamber. The output was monitored by a calibrated directional coupler and power meter (Hewlett-Packard 423A). A leveling loop circuit from the meter back to the amplifier was established to maintain a constant level of transmitted power.

Pulsed microwave exposures were produced by a 5 KW pulsed microwave generator (Applied Microwave Laboratory Model PG5), coupled to the same standard gain horn used for CW radiation. Output was monitored by the same calibrated directional coupler and power meter as in the CW case. The average rms power was monitored by the power meter and pulse wave shape, pulse width, and pulse repetition frequency by oscilliscope (Tektronic 454) from monitor jacks provided on the PG-5 pulsed transmitter. All pulsed transmissions were of square wave form.

The system was calibrated through the use of the standard gain horn to calculate the field intensity in the far field with a dipole antenna as a transfer standard. The field intensity was further checked with both a NBS field intensity meter and a Narda Model 8300 isotropic radiation monitor. Overall accuracy of reported peak and average power density measurements is estimated to be better than ± 20%.

The rats were exposed individually for 20 minutes on a styrofoam pedestal. One of two different exposure procedures was used. In the recovery time study, the rats were irradiated without anesthesia. The rats were placed in a pie shaped, well ventillated, styrofoam enclosure. The box was placed on the styrofoam platform with the rat facing the center of the emitting horn and aligned with the longitudinal axis of the horn. The rat could move his head and lick his paws but could not turn around or move laterally. The animals were then anesthetized at 8 min, 4 hrs, or 24 hrs, after exposure, injected with the radiolabeled test substances, and sacrificed.

In all other tests the animals were anesthetized before microwave exposure. The anesthetized rat was placed directly on the styrofoam platform facing the mouth of the horn and aligned with the longitudinal axis of the horn. After the 20 min microwave exposure, the rats were then injected with the test substance and sacrificed, using the same procedure as above. The elapsed time from when the animals were anesthetized, exposed, injected, and sacrificed was never more than 40 minutes.

The method used to measure the brain uptake of radiolabeled substances was based on Oldendorf's double indicator technique which uses a highly diffusible substance as a reference standard (21,22). Two labeled indicators were injected simultaneously. Tritiated water, ^{3}HOH, was used as the diffusible internal reference standard. The other indicator was the test substance under study and was labeled with ^{14}C. The procedure is summarized below.

A mixture containing approximately 0.75 μc of the labeled test substance and 1.0 μc of ^{3}HOH (SA=0.25 mc/g) was used with a diluent to make up a total volume of 0.2 ml solution. The diluent was a Ringer's solution (105ml - H_2O, 20 ml - 4.5% NaCl, 0.8 ml - 5.75% KCl, 0.6 - ml 6.1% $CaCl_2$ 0.4 ml - 6.7% $k_2 PO_4$, 0.2 ml - 10.55% $KH_2 PO_4$, and 4.2 ml - 6.5% $NaHCO_3$) buffered to pH 7.55. Such a solution is preferable to rat serum because it eliminates the possibility of tracers binding to the serum protein or substances in the serum competing with the tracers for transport. The test substances used in this study were (^{14}C) D-Mannitol (SA=10-150 mc/mM) with a molecular weight (MW) of 182.2, (^{14}C)-Inulin (SA=2-3 mc/mg) with MW 5,000 - 5,500, and (^{14}C)-Dextran (SA=0.5-2 mc/g) with MW 60,000 - 75,000. Isotopes were from ICN Isotope & Nuclear Division, Cleveland, Ohio or New England Nuclear, Boston, Massachusetts.

The rats were anesthetized with intraperitoneal pentobarbital (I.P., 60 mg/kg). The right common carotid artery was surgically exposed and cannulated using a 27-gauge needle. The needle did not occlude the vessel, and free arterial flow past the needle persisted throughout. The entire 0.2 ml of solution (temperature 22-25°C) was injected rapidly. The needle was left in the artery after injection to prevent excessive bleeding. The artery became clear during injection and blood flow was normal immediately after. The animal was sacrificed by decapitation 15 sec after injection.

The whole brain was quickly dissected free and the following tissue sections were placed rapidly into scintillation vials: cortex, hippocampus, hypothalamus, cerebellum, and medulla. Entire brain areas or large sections of brain areas were used to maintain consistency between animals and to guard against the possibility of using one area of the cortex in one animal and a different area of the cortex in another animal, where uptake may vary. For example, the whole hypothalamus was always taken, the third of the cerebellum ipsilateral to injection was taken, the fourth of the cortex ipsilateral to injection and rostral to the midbrain were taken to provide greater consistency between animals. The tissues were digested overnight at room temperature with 1 ml aliquots of a quaternary ammonium hydroxide tissue solubilizer (Soluene 350, Packard). Ten ml aliquots of liquid scintillation mixture (Dimilume - 30, Packard) were added to each vial and the samples were subjected to routine scintillation counting. Several samples of the injected mixture were subjected to the same procedure. Radioactivity was measured with a Beckman LS-355 liquid scintillation counter which was equipped with an external standard and automatic quench control for counting dual isotope samples. The raw counts (c.p.m.) for each isotope were converted to the absolute rate of disintegration (d.p.m.) using quench and background curves and solving two simultaneous equations.

Following the procedure of Oldendorf, the ratio of ^{14}C of the test substance to ^{3}H of the diffusible standard in the brain tissue is divided by the same ratio in the respective injection mixtures. This ratio is presented by Oldendorf as the Brain Uptake Index (BUI) and is defined as:

$$BUI = \frac{^{14}C/^{3}H \text{ in brain Tissue}}{^{14}C/^{3}H \text{ in injected mix}} \times 100$$

This ratio of ^{14}C to ^{3}H in brain tissue relative to the ratio of ^{14}C to ^{3}H in the original injected mixture defines the relative amount of test substance lost to the brain in a single passage through the microcirculation. The ^{3}HOH which enters the brain distributes in the course of one capillary passage with most of it leaving the brain capillaries. The amount of ^{3}HOH entering the brain tissue is flow dependent and defines the amount of

injected bolus which pases through the piece of tissue examined. Some fraction of the labeled test substance also leaves the blood and enters the brain tissue. The remaining fraction is not taken up during a single passage and is carried out of the brain blood compartment before sacrifice. This method corrects for regional differences in blood flow and should determine whether an increase in a labeled test substance is due to a blood-brain barrier alteration or to blood flow or to both. If the test substance had the same uptake as water, the BUI would be 100. If the test substance did not leave the blood vessels at all, the BUI would be zero. In practice, even neutral polar substances which exhibit negligible penetration of the BBB during a single capillary passage, have a BUI of 1 to 3. This number can be interpreted as a background level, and is due perhaps either to the fact that not all of the test substance is completely washed out of the brain blood compartment, or to some recirculation of test substance, or to some of the substance adhering to the inner surface of the capillary endothelium.

Inital procedures were designed as a double blind evaluation with the inclusion of blanks. One investigator selected the animals, coded them, either anesthetized them or put them in the exposure box, placed them in the anechoic chamber and choose the irradiation condition. The second investigator was given the animal without knowledge of the exposure condition. This second investigator injected the rat with the radioisotopes, sacrificed the animal, and placed the brain tissue sample in scintillation vials with a new code. A third investigator had the vials' radioactive level counted and determined the BUI for each vial. Only then were the three codes compared to determine the BUI corresponding to each animal's exposure condition and brain tissue area.

RESULTS

The first test series was designed to detect a measurable permeability change in rats due to microwave exposure. D-mannitol was chosen as the test substance because of its low molecular weight, 182.2, and because it does not normally cross the BBB system. The rats were randomly selected and individually exposed for 20 minutes to one of three irradiation conditions: sham irradiated; 1.3 GHz pulsed microwaves with a 10 microsecond (μs) pulse width, a pulse repetition frequency (prf) of 1,000, an average power density of 2.0 milliwatts per square centimeter (mW/cm^2), and a peak power density of 200 mW/cm^2; and 1.3 GHz pulsed microwave with a 10 μs pulse width, 50 prf, 0.3 mW/cm average power density, and 600 mW/cm^2 peak power density. There were 5 rats in each test cell and blanks were introduced.

Figure 1 shows the results of the first test series in terms of the brain uptake index (BUI) for the three exposure conditions and five different brain regions. Each bar represents the mean of five animals. The error bars are the standard deviation of the mean and the p values are the statistical significance of the difference between means of the microwave irradiated groups and the controls using the Student's t-test. The largest changes in uptake occurred in the medulla, followed by the cerebellum and hypothalamus. The BUI for the controls went from about 1.5 for the hippocampus to 3.2 for the medulla. For the 2.0 mW/cm^2 average power density case, the BUI varied from about 1.2 times the control value for the cortex to 2.9 times the value of the controls for the medulla. In the 0.3 mW/cm^2 average power density case, the BUI of the microwave irradiated rats varied from 1.5 times the controls in the cortex to 3.7 times the value of the controls in the medulla.

The second test series was conducted to determine the effects of microwaves on the permeability of several neutral polar substances of different molecular weights. Three different substances were chosen: D-mannitol with a molecular weight of 182.2, because it is small and was known to be very sensitive to BBB alterations; inulin with a MW of 5,000, which is similar in weight to many unbound dyes which are used for tracers; and dextran with a MW of 60,000 to 75,000, which would be similar in weight to dye-protein complexes. All of these substances have a negligible uptake in brain tissue under normal conditions.

Figure 2 shows the results of this test series. The controls were sham irradiated for 20 minutes. The exposed animals were irradiated with 1.3 GHz, pulsed microwaves with a 0.5 μs pulse width, 1000 prf, 0.3 mW/cm^2 average power power density, and 600 mW/cm^2 peak power density for 20 minutes. The tests a resulted in statistically significant permeability increases in the hypothalamus, cerebellum, and medulla for both the mannitol and inulin. With dextran there were negligible changes, with only the cerebellum showing a difference at the 0.05 level of significance.

The third test series was performed to determine the duration of the BBB alteration, and whether or not there is recovery. In this test series only, the alternative test procedure was used where the animals were exposed without anesthesia in a specially designed enclosure. After exposure, the animals were put back into their cages. The animals were then anesthetized, injected with the mannitol test mixture, and sacrificed at three different times after exposure. Separate controls (sham irradiated) were used with the microwave irradiated animals at each post-irradiation, interval.

The results of the third test series are shown in figure 3. The rats were sacrificed 8 minutes, 4 hours, or 24 hours after either 20 minutes of sham irradiation or 20 minutes of 1.3 GHz, 1,000 prf, 0.5 μs pulse width, 0.3 mW/cm^2 average power density, 600 mW/cm^2 peak power density exposure. The 8 minute and 4 hour groups were very much the same and showed statistically significant increases in BBB permeability over controls. By 24 hours, the uptake of mannitol was almost back to normal.

The next test series was conducted to determine if CW microwave energy would affect the uptake of mannitol and if so, to compare the magnitude of change to that produced by pulsed microwave irradiation. Also, the functional relations between average power density and BBB permeability increases produced by different microwave modulation conditions were explored. One hundred and five male Wistar rats served as subjects. They were exposed to one of 19 different irradiation conditions for 20 minutes: sham irradiated; 10 different 1.3 GHz, CW microwave exposure conditions with power density from 0.3 mW/cm^2 to 3.1 mW/cm^2; 6 different 1.3 GHz, 1000 prf, 0.5 μs, pulsed microwave conditions of average power density 0.1 mW/cm^2 to 0.8 mW/cm^2; and 2 different 1.3 GHz, 5 prf, 10 μs, pulsed microwave conditions of average power density 0.03 to 0.05 mW/cm^2.

Figures 4 and 5 give the results for the cerebullum and medulla. The uptake of mannitol was a very definite function of exposure parameters. For the CW microwave case, the uptake of mannitol increased with increasing power density up to about 1.0 mW/cm^2 and then started to decrease. The pulsed microwave cases produced similar changes but at different average power densities. There was a definite difference (statistically significant at the overlap points of 0.3, 0.4 and 0.6 mW/cm^2) between the permeability change produced by the CW and pulsed microwaves of the same average power density. There was also a definite difference in mannitol uptake produced by pulsed microwaves of the same average power density but different pulsed characteristics.

The graphs also show that there was a statistically significant increase in the uptake of mannitol in the medulla at an average power density of only 0.03 mW/cm^2. The results for the hypothalamus were similar, but there was less of a magnitude of change. The cortex and hippocampus results were erratic and changes in permeability were small.

Table 1 is a summary of the pulsed microwave data from several of the test series. It lists the BUI for the hypothalamus, cerebellum and medulla as the mean of 3 to 13 animals plus or minus the standard deviation of the mean. All data are for 20 minute exposures at a carrier frequency of 1.3 GHz. The values for the sham irradiated animals are about 2.2 for the hypothalamus, 2.5 for the cerebellum and 3.4 for the medulla. Three primary microwave parameters (pulses per sec, pulse width, and peak power density) are denoted as x_1, x_2 and x_3. Other microwave parameters (except frequency and exposure time, which were fixed) are secondary parameters and are derivable from x_1, x_2, and x_3. For example, the table lists average power density, which is x_1 times x_2 times x_3, and energy per pulse which is x_2 times x_3. There is not enough data to rigidly determine the functional dependence of mannitol uptake on the respective microwave parameters, but certain trends are suggested. Lines 1 and 12 in table 1 have x_1 and x_3 constant and x_2 varied. This suggests that as the pulse width is increased the mannitol uptake goes up. Similarly numbers 7 and 9 in the table indicate that as the number of pulses per second increases the BUI also goes up. Numbers 1 through 8 in the table indicate that as the peak power density is increased (up to about 0.8 mW/cm^2) the magnitude of the permeability change is also increased.

The data in the table suggest that the BUI is a function of peak power density, pulse width, and the number of pulses per second. The effect of peak power on the uptake of mannitol seems greater than the effect of pulse width and both seem to have a greater influence on permeability than the number of pulses per second. This implies that for a given average power density one can get a higher permeability change by raising the peak power and lowering the number of pulses per second than by raising the pulses per second and lowering the peak power. Such phenomena would give rise to a family of curves of BUI versus average power which vary depending on the x_1, x_2 and x_3 characteristics. Figures 4 and 5 give preliminary indications of such a family of curves.

DISCUSSION

The results of the present study indicate that low power, pulsed and CW microwave exposures affect brain tissue permeability, thus confirming Frey's findings (17). It does not appear that pulsed energy is always more effective in causing BBB alterations than CW energy, but that depending on certain pulsing characteristics, pulsed energy can be more or less effective than CW energy of the same average power density. From figures 4 and 5 it can be observed that pulsed energy with high peak power, large pulse widths, and few pulses per second effected the uptake of mannitol at an average power density of only 0.03 mW/cm^2, where it took CW energy of approximately 0.3 mW/cm^2 to cause the same magnitude of change.

In general, the uptake of mannitol increased with increasing power density for both pulsed and CW microwave signals up to 0.5 to 2.0 mW/cm^2, where the BUI started to level off and then, decrease. A similar amplitude "tuning curve" or inverted U shaped function has been observed by Bawin and Adey for calcium efflux with weak low frequency electric fields of similar amplitude (9). Equipment used in the present study did not allow for power density exposures above 3.1 mW/cm^2, so it is not known whether the permeability

change continues to decrease or rise again as the power is increased further. Such a complex function may be the reason that other researchers have not observed BBB alterations, as the tendency of most researchers is to start at high power levels looking for an "effect" and then to work down in power.

Exposed and sham irradiated rats exhibited similar regional differences in the uptake of the three neutral polar substances in the present experiments. The magnitude of the uptake of the radiolabeled substances was lowest in the cortex and hippocampus, with higher uptakes in the hypothalamus, followed by the cerebellum and the highest in the medulla. These regional differences agree with recent literature, but are difficult to interpret (15,23). The cerebellum and medulla, which contain the area postrema, always exhibited more uptake than the hypothalamus, which is considered to be an area of diminished BBB. In turn, the uptake of the hypothalamus was similar to that of the hippocampus and cortex, areas of supposedly, uniformly well developed BBB. The short interval (15 sec) between isotope injection and sacrifice, was felt to minimize the distribution and spread throughout the brain of any tracer that had entered the brain tissue. Perhaps the BBB is more developed in the hypothalamus than previously thought or perhaps the site of leakage is concentrated in the area postrema. In experiments with cold stress, brain concussions, and ionizing radiation, it has been observed that the brain stem and upper cervical cord are the regions of the CNS which are most effected (15,16,24).

There is a remote possibility that the increase in BUI produced by microwave exposure occurs because of the reduced uptake of tritiated water, rather than because of the increased uptake of mannitol and inulin. The lack of change in the dextran uptake due to microwave exposure mitigates this possibility and reinforces our conclusion of changes in the permeability of the BBB to mannitol and inulin. In the present study, dextran, which is similar in molecular weight to protein, did not penetrate the barrier, but inulin, which is similar in molecular weight to many dyes used as tracers did. This suggests that in earlier microwave studies, which used dyes as tracers, a penetration of unbound dye was being observed rather than a penetration of dye-protein complexes.

Microwaves can be characterized by four fundamental or first order parameters; frequency, pulse width, pulse repetition frequency, and peak power density (amplitude). Other microwave parameters such as average power density, energy per pulse, total energy, duty cycle, etc. are second order parameters and are derivable from the four fundamental ones. Dealing with secondary parameters, or a mixture of secondary and primary parameters, may not uncover functional dependencies. For example, in holding average power density constant and varying peak power density, the average power density is in fact derivable from three primary parameters, one of which is peak power density. Several investigators in an effort to determine the cause or at least the functional dependence of biological effects, for example r.f. sound, have conducted numerous experiments holding some of the secondary parameters constant and varying others (17,25). It seems that a more rigorous approach, and the one attempted in this study, would be to isolate the primary microwave parameters and investigate their effect on the biological phenomena. With this type of analysis, the functional dependence of brain permeability on the microwave parameters can be examined.

In 1961, the same year Frey discovered r.f. sound, White independently demonstrated that pulsed electromagnetic energy could induce pressure pulses in material (26,27). As further developed by White and Gournay, it was shown that these elastic stress waves were a function, for a single

pulse, of peak power density for long pulse widths and peak power density times pulse width for short pulse widths (28,30). Since in the present study CW microwaves were very effective in creating BBB alterations, it would seem unlikely, even though pressure pulses and brain concussions are known to cause BBB alterations, that such a phenomenon is the major cause of the permeability change. Another possibility may be local heating due to"hot spots" or focusing of energy, since the greatest BBB alteration occurs in the cerebellum and medulla or close to the neck region of rats. A theoretical prediction of such neck heating has been made by Gandhi (31,23). Trace metal content and neurotransmitter changes have also been observed due to whole body heating and to 10 minute exposures at an average power density of 80 mW/cm^2, 1.6 GHz microwave energy (33,34). This work demonstrated large amplitude power deposition in the floor of the brain of rats. It was hypothesized that microwave induced hyperthermal environments were the cause. Because of the lower powers that were involved in the present study, 0.03 mW/cm^2 average power density for pulsed microwaves and 0.3 mW/cm^2 for CW microwaves, the possibility of the BBB alteration being caused by direct heating seems remote. The present data do not address the question of whether the microwave exposure interacts directly to alter the BBB system or whether the microwave exposure causes an indirect effect. The data also do not address the currently debated question of whether BBB alterations are due to lesions or increases in micropinocytotic vesicle transfer (35-38). Future work is planned to address these questions.

Comparison of the uptake of mannitol for the case of sacrifice 8 minutes after irradiation, figures 2 and 3, demonstrates that neither the head movement nor the anesthesia seems to affect the uptake level of the sham or microwave irradiated rats. With anesthesia, the animals were always facing the emitting horn and were immobile. Without anesthesia, the animals were free to move their head and paws within their enclosure. Both exposure procedures resulted in similar permeability changes.

Recent findings with several independent confirmations have demonstrated that low power microwaves can interact with animals to cause CNS changes such as auditory sensations, calcium efflux changes, regional histopathology, and altered EEG patterns (4-9). This paper demonstrating blood-brain barrier alterations is another major example of CNS changes due to microwaves at average power densities below 10 mW/cm^2. It should be emphasized, however, that no one has determined whether or not these CNS changes are hazardous. Possibly, they may even be beneficial. For example, selective BBB changes may enhance the permeability of therapeutic pharmacological agents.

SUMMARY AND CONCLUSIONS

Single, 20 minute exposures to pulsed or CW microwave energy at 1.3 GHz induced an increase in the uptake of D-mannitol in rat brain tissue. Permeability changes were observed at average power densities as low as 0.03 mW/cm^2 with pulsed microwaves, and as low as 0.3 mW/cm^2 with CW signals. The BBB alterations were greatest in the medulla, followed in decreasing order by the cerebellum, hypothalamus, hippocampus, and cortex. Increases in permeability were observed for radiolabeled mannitol and inulin, but not for dextran. The permeability changes for mannitol occurred 8 minutes and 4 hours after pulsed microwave exposure, but not after 24 hours. Differences in the level of uptake were noted between CS and pulsed microwaves of the same average power density and also between pulsed microwaves of different modulation characteristics but with the same average power density. The findings suggest that microwave energy induces a temporary

change in the permeability for small neutral polar molecules in the blood-brain barrier of rats.

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39. The opinions or ascertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Army.

Fig. 1. Brain uptake of ^{14}C-mannitol for 3 irradiation conditions and 5 different brain regions. Each bar represents the mean ± S.D. of the mean for 5 animals. The p- values represent the statistical significance that the mean of the microwave irradiated animals is different than the sham irradiated ones.

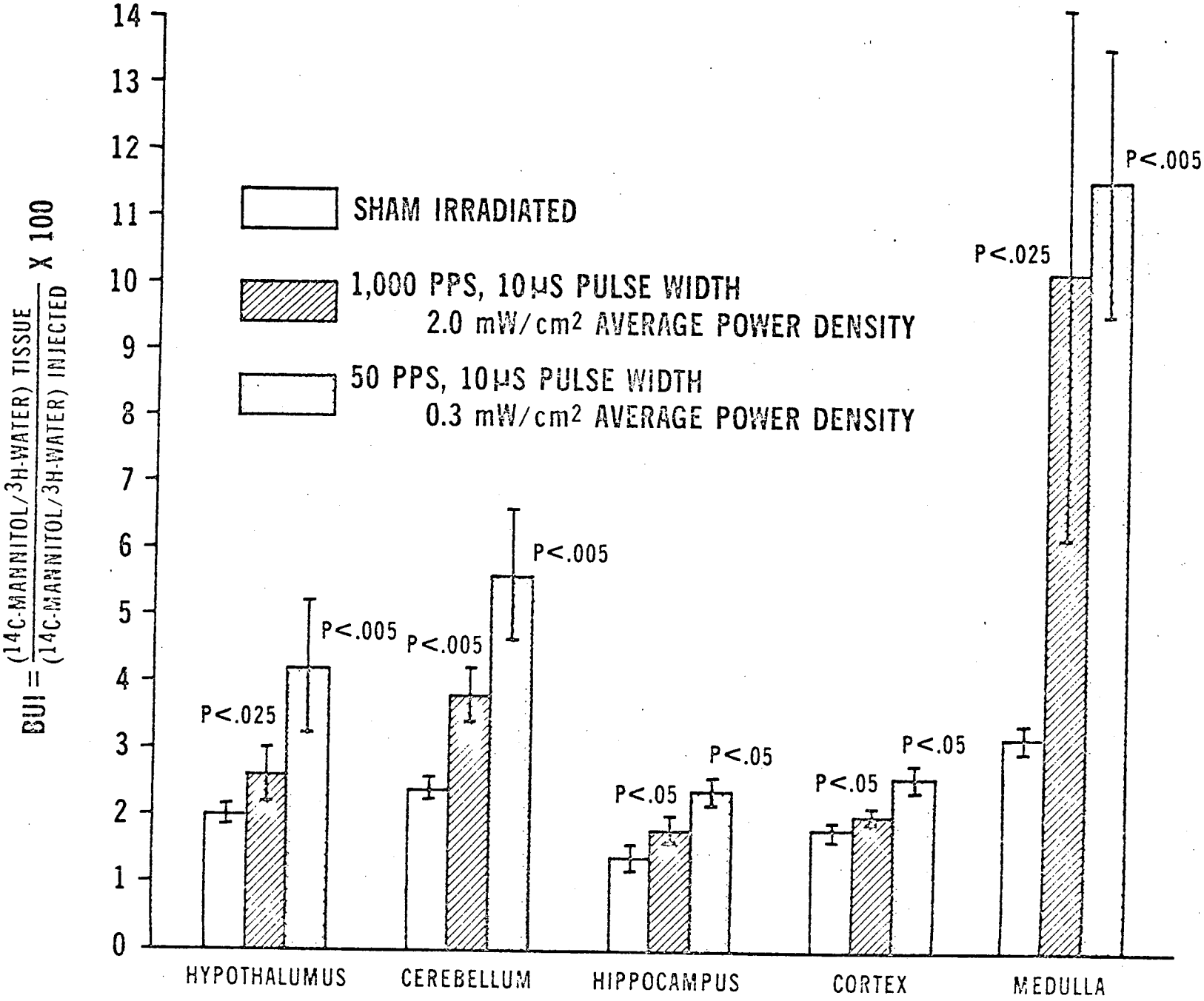
Fig. 2. Brain uptake of mannitol, inulin, and dextran for 5 different brain regions in controls and exposed animals. Controls were sham irradiated and exposed were irradiated for 20 minutes with 1.3 GHz, 1000 prf, 0.5 μs, pulsed microwaves of 0.3 mW/cm^2 average and 600 mW/cm^2 peak. Each bar is the mean ± S.D. of 5 rats. The p- values repesent the statistical significance that the exposed are different than the controls.

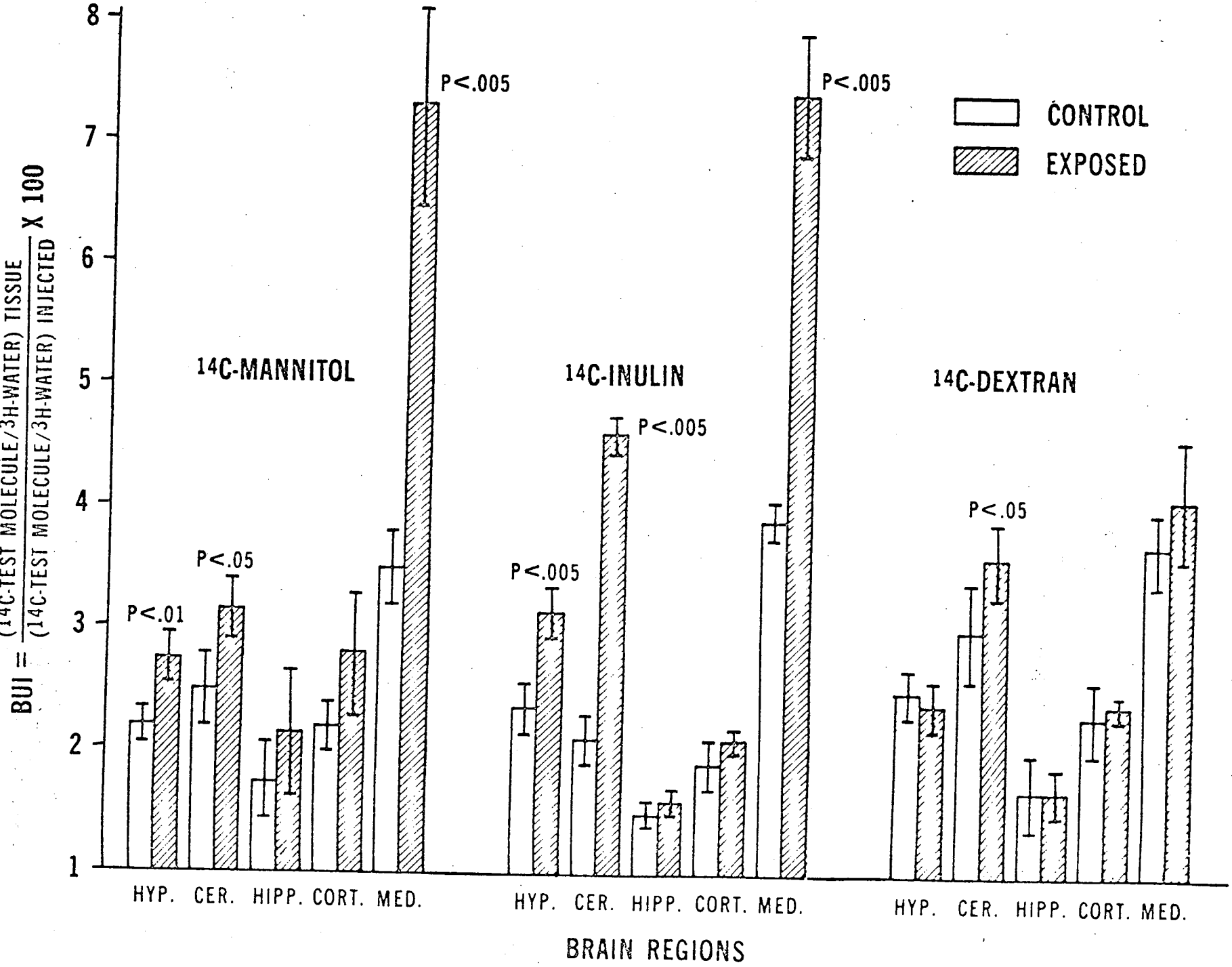
Fig. 3. Brain uptake of mannitol 8 minutes, 4 hours, and 24 hours after sham irradiated (control) or exposed to 1.3 Ghz, 1000 prf, 0.5 μs, pulsed microwaves of 0.3 mW/cm^2 average power density and 600 mW/cm^2 peak power density for 3 different brain regions. Each bar represents the mean ± S.D. of 5 animals and the p- values are the statistical significance that exposed are different than the controls.

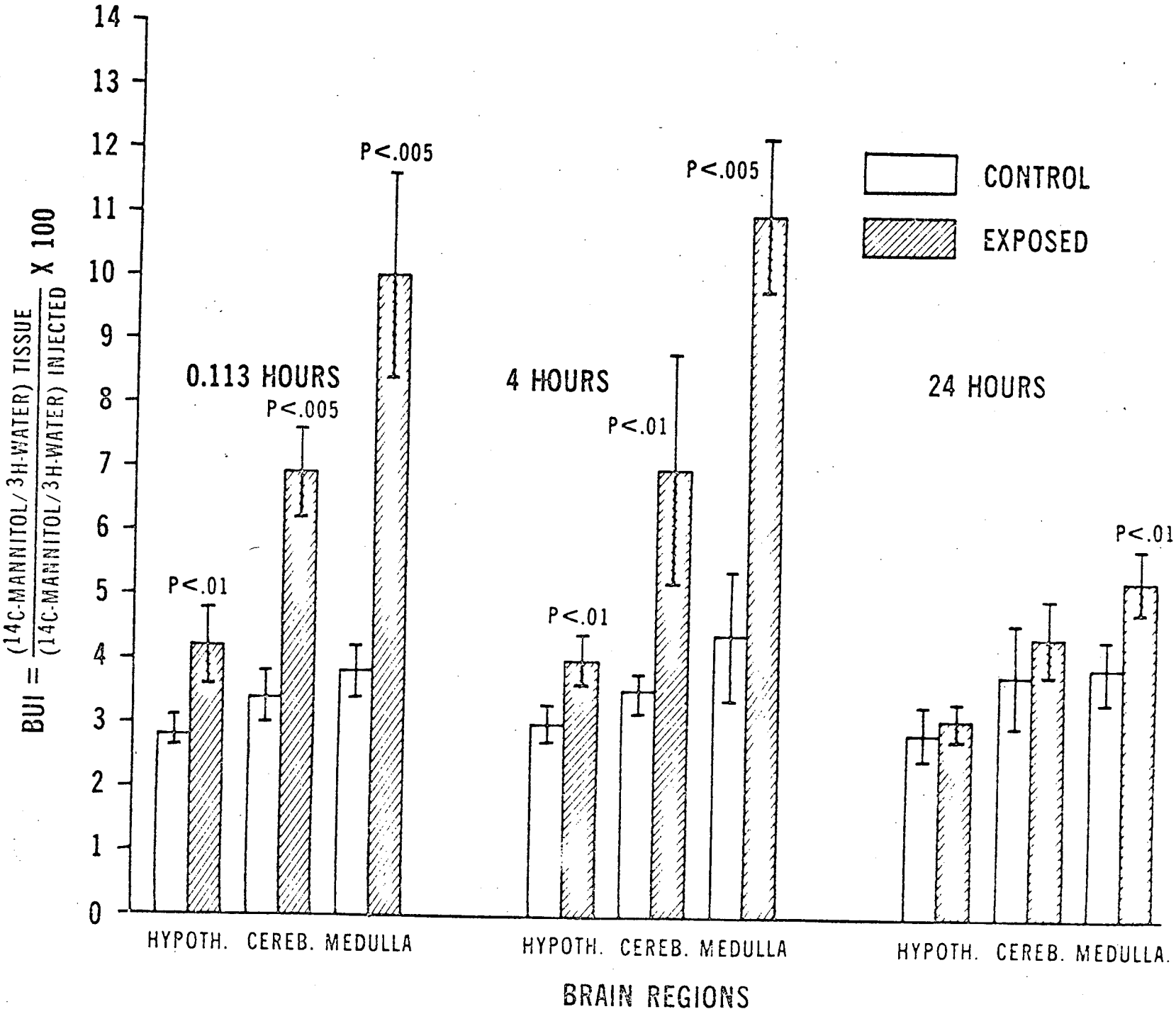
Fig. 4. Uptake of mannitol in the cerebellum as a function of average power density for 3 types of microwave irradiation. Each point represents the mean ± S.D. of 3 to 13 rats.

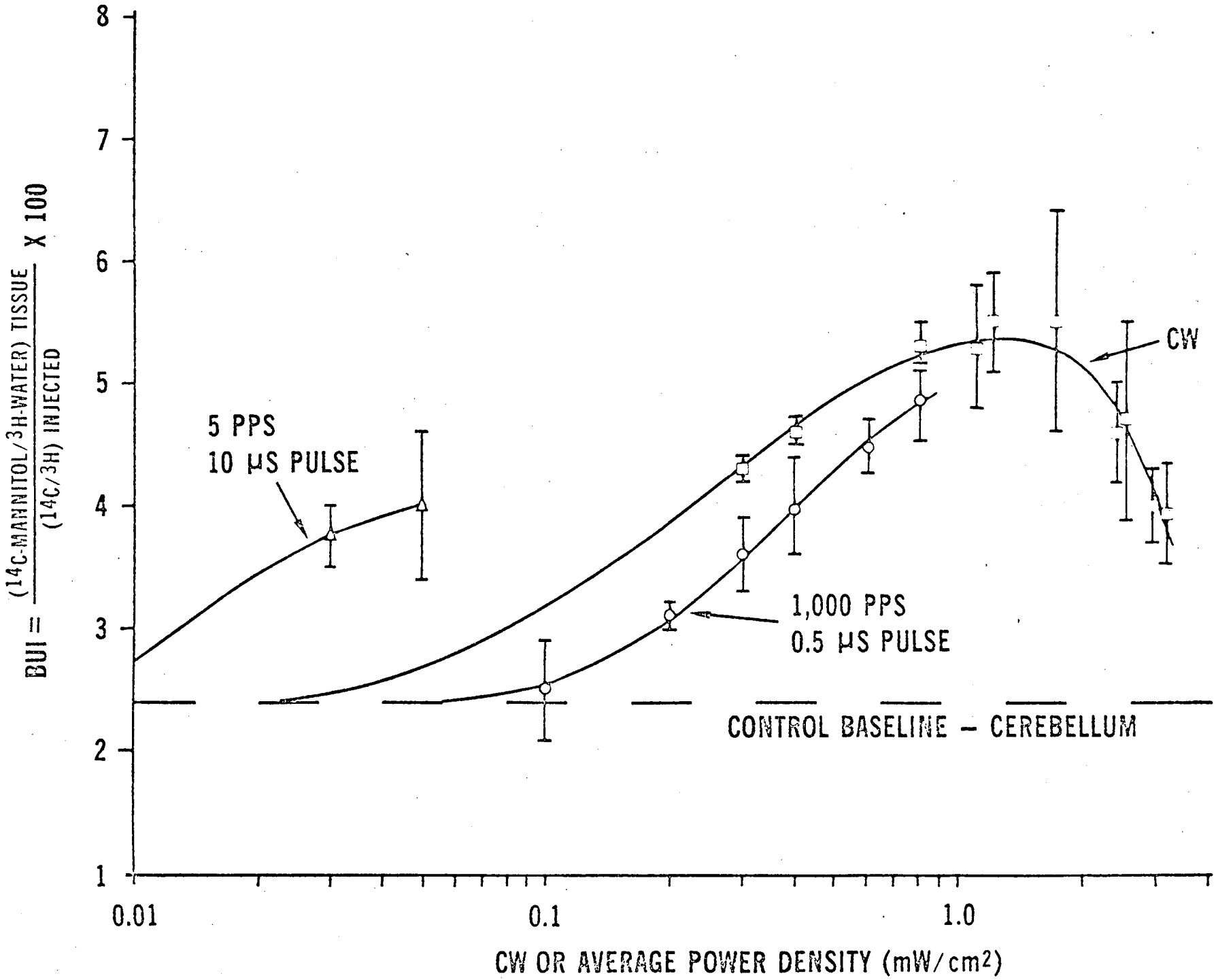
Fig. 5. Uptake of mannitol in the medulla as a function of average power density for 3 types of microwave irradiation. Each point represents the mean ± S.D. of 3 to 13 rats.

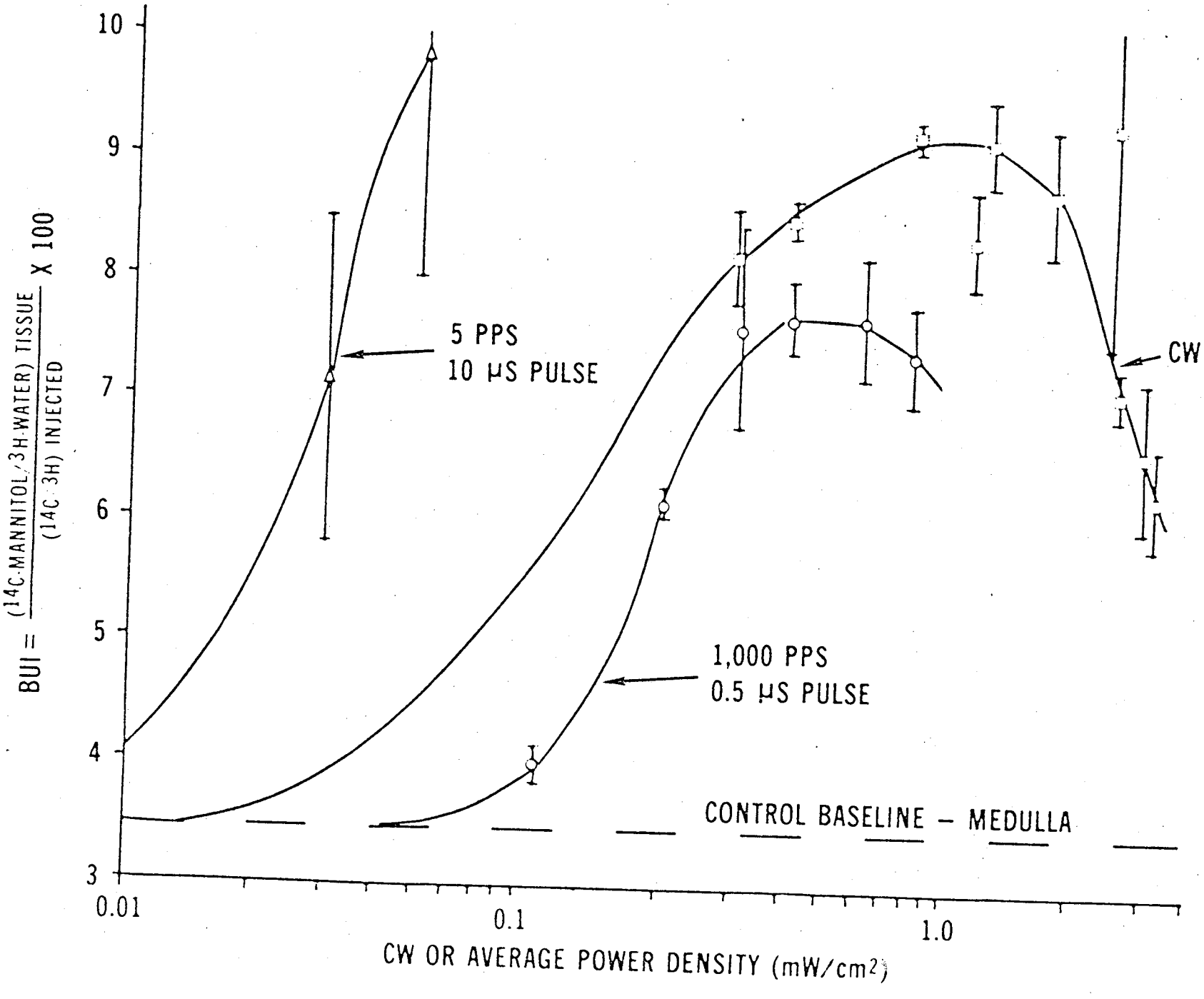
TABLE 1. Uptake of ^{14}C-mannitol for 12 different 1.3 GHz, 20 minute, pulsed microwave exposure conditions and 3 different brain regions. Each BUI value represents the mean ± S.D. of 3 to 13 animals. The value for controls is approximately 2.2 for the hypothalamus, 2.5 for the cerebellum, and 3.4 for the medulla.


BUI = (14C-MANNITOL/3H-WATER) TISSUE / (14C-MANNITOL/3H-WATER) INJECTED X 100
SHAM IRRADIATED
1,000 PPS, 10μS PULSE WIDTH
2.0 mW/cm2 AVERAGE POWER DENSITY
50 PPS, 10μS PULSE WIDTH
0.3 mW/cm2 AVERAGE POWER DENSITY
P<.025
P<.005
P<.005
P<.005
P<.05
P<.05
P<.05
P<.025
P<.005
HYPOTHALUMUS
CEREBELLUM
HIPPOCAMPUS
CORTEX
MEDULLA
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14


BUI = (14C-TEST MOLECULE/3H-WATER) TISSUE / (14C-TEST MOLECULE/3H-WATER) INJECTED X 100
8
7
6
5
4
3
2
1
14C-MANNITOL
14C-INULIN
14C-DEXTRAN
P<.01
P<.05
P<.005
P<.005
P<.005
P<.005
P<.05
CONTROL
EXPOSED
HYP. CER. HIPP. CORT. MED.
HYP. CER. HIPP. CORT. MED.
HYP. CER. HIPP. CORT. MED.
BRAIN REGIONS


BUI = (14C-MANNITOL/3H-WATER) TISSUE / (14C-MANNITOL/3H-WATER) INJECTED X 100
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0.113 HOURS
4 HOURS
24 HOURS
P<.01
P<.005
P<.005
P<.01
P<.01
P<.005
P<.01
CONTROL
EXPOSED
HYPOTH. CEREB. MEDULLA
HYPOTH. CEREB. MEDULLA
HYPOTH. CEREB. MEDULLA.
BRAIN REGIONS


BUI = (14C-MANNITOL/3H-WATER) TISSUE / (14C/3H) INJECTED X 100
8
7
6
5
4
3
2
1
0.01
0.1
1.0
CW OR AVERAGE POWER DENSITY (mW/cm2)
5 PPS
10 μS PULSE
1,000 PPS
0.5 μS PULSE
CW
CONTROL BASELINE – CEREBELLUM


BUI = (14C-MANNITOL/3H-WATER) TISSUE / (14C 3H) INJECTED X 100
10
9
8
7
6
5
4
3
0.01
0.1
1.0
CW OR AVERAGE POWER DENSITY (mW/cm²)
5 PPS
10 µS PULSE
1,000 PPS
0.5 µS PULSE
CW
CONTROL BASELINE – MEDULLA

MICROWAVE PARAMETERS						BRAIN UPTAKE INDEX		
NO.	X_1 PULSES/SEC.	X_2 PULSE WIDTH (μS)	X_3 PEAK PD. (mW/cm^2)	$X_1X_2X_3$ AVE PD. (mW/cm^2)	X_2X_3 ENERGY/PULSE ($\mu J/cm^2$)	HYPOTHALUMUS	CEREBELLUM	MEDULLA
1	1,000	0.5	200	0.1	0.1	2.3 ± 0.1	2.5 ± 0.4	4.0 ± 0.1
2	1,000	0.5	400	0.2	0.2	2.4 ± 0.2	3.1 ± 0.1	6.2 ± 0.1
3	1,000	0.5	600	0.3	0.3	2.9 ± 0.4	3.6 ± 0.6	7.6 ± 0.9
4	1,000	0.5	800	0.4	0.4	2.8 ± 0.1	4.0 ± 0.5	7.7 ± 0.5
5	1,000	0.5	1,200	0.6	0.6	3.2 ± 0.1	4.5 ± 0.3	7.7 ± 0.9
6	1,000	0.5	1,600	0.8	0.8	3.3 ± 0.2	4.9 ± 1.3	7.4 ± 0.5
7	5	10.0	600	0.03	6.0	3.0 ± 0.1	3.8 ± 0.2	7.2 ± 1.7
8	5	10.0	1,000	0.05	10.0	3.5 ± 0.6	4.0 ± 0.7	9.8 ± 4.2
9	50	10.0	600	0.3	6.0	3.9 ± 1.3	5.8 ± 2.1	10.0 ± 5.7
10	250	20.0	60	0.3	1.2	3.3 ± 0.4	4.2 ± 0.6	7.0 ± 0.3
11	250	2.0	600	0.3	1.2	4.5 ± 1.2	5.1 ± 1.8	7.5 ± 2.1
12	1,000	10.0	200	2.0	2.0	2.6 ± 0.4	3.8 ± 0.5	9.1 ± 4.2