Study of the Use of the Microwave Magnetic Field for the Rapid Inactivation of Brain Enzymes

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Abstract—A new model of a microwave device was developed with a power of 10 kW at 2450 MHz. In order to accomplish even distribution of heating with minimum trauma and with a maximum certainty about enzyme inactivation, a modified magnetic field distribution was utilized rather than the conventional electric field. An integrated tuning system was used to increase efficiency and distribution of microwave energy absorption. This increased the ability of the instrument to properly inactivate the enzymes in the brain of both mice and large rats. In general, the time of irradiation for the rat was 600 to 900 msec and for the mice, 100 to 330 msec. The animal chambers used were designed so as not to impair breathing or too severely restrict movement. The effects of these improvements on microwave irradiation were confirmed by 1) observation of brain appearance, 2) effects on succinic dehydrogenase and cholinesterase activity, 3) measurement of regional temperatures in the animal's brain, 4) thermograms of the brain, 5) electron microscopic examination of brain tissue and 6) measurement of endogenous acetylcholine and catecholamines.

The technique of microwave irradiation (MWR) for the inactivation of brain enzymes of animals has been widely used since its introduction in 1970 (1). It has gained widespread acceptance because it is a sacrifice method which makes possible sampling and measurement of many enzymatically destroyed brain neurochemicals by inactivating the enzymes, lessening the influence of postmortem changes (2, 3).

Discussion of the method and its application has been the subject of several reviews (4–6) and scientific reports (5–30). Many of these results have been recently summarized in the Proceedings of the Symposium on “Drug Effects on Rapidly Metabolized Compounds in the CNS: Rapid Tissue Fixation with MWR (1981)” which was held at a Satellite Symposium of the 8th International Congress of Pharmacology in Tokyo, 1981 (10).

However, there is still misunderstanding about the application of microwave fixation not because of doubt about the usefulness of the technique, but because of some unsuccessful applications. These errors seem to occur primarily because of the nonuniformity of heating which results in incorrect sampling and assay of levels of endogenous compounds (29, 31). A primary reason for these problems most likely lies in the use of inadequately controlled microwave devices. Use of various simple microwave devices which do not have adequate control and adjustment mechanisms has given rise to a multitude of problems related to the uniformity of heating, residual enzyme activity, coupling efficiency of the device, stress-induced changes in brain chemistry and compound translocations associated with tissue disruption. Heating brain tissue by microwave energy makes it possible to rapidly heat
the brain and inactivate enzymes; however, it does not necessarily provide uniform distribution of the heat or the ability to repeat the same distribution in subsequent use. (6, 10, 32).

To organize a system to obtain proper distribution of tissue heating, a certainty of enzyme inactivation with minimum trauma and retention of valid labile compound concentration, the following criteria should be addressed:

**The instrument:** 1. Must provide a wide distribution of power in the brain. 2. Must have adequate power and coupling efficiency to heat the brain rapidly. 3. Should have a rapid rise time, that is, the time required for full power to be delivered to the tissue should be a small segment of the firing interval. 4. Must be readily tuneable so that the power distribution can be optimized for variation in animals.

**The animal:** 1. Should be comfortable in the container. 2. Should not be unduly restrained. 3. Should not be detained in the container or apparatus for more than a few seconds before sacrifice.

**The tissue:** 1. Upon dissection, following microwave heating, the brain should appear hardened and intact with a uniform, grey appearance with no apparent bloody areas. 2. Must have been heated over a wide area with the adequacy of the distribution reflected by: a. A relatively even temperature distribution, as measured immediately following heating. b. A widespread enzyme inactivation which occurs very early during power application: that is, most heating occurs by primary power deposition and not conduction from heated to non-heated tissue. 3. Concentration of enzymically labile compounds whose action is rapidly terminated by enzyme action should yield different values following microwave irradiation sacrifice and sacrifice by decapitation.

In order to satisfy these criteria for successful inactivation of brain enzymes in rat and mouse, an instrument has been developed for applying the magnetic field (H-field) with a tuning system.

In this paper, we describe the NJE2603 system with a 10 kW input operating at a frequency of 2450 MHz and discuss its usefulness in relation to the listed criteria.

**Materials and Methods**

**Animals:** Male Sprague-Dawley rats (250–420 g) purchased from Nihon Clea were used. Rats were housed two rats per cage for at least 7 days before use. The mice were obtained from Nihon Clea and weighed 18–28 g. The animals were maintained at a temperature of 23±1°C and a humidity of 55±5%. The light cycle was from 7 a.m. to 7 p.m. The animals were allowed constant access to water and standard laboratory chow (Nihon Clea, Tokyo, Japan).

**Material:** All the chemicals employed were reagent grade, obtained from either Wako Pure Chemical Industries (Tokyo, Japan) or Sigma (St. Louis, MO, U.S.A.).

**Construction details of the microwave instrument:** The diagram of the mechanical structure of the equipment is shown in Fig. 1. The structure consists of four main parts: 1. Power source, control unit, high voltage unit and magnetron. 2. Isolator. 3. Tuning system. 4. Exposure chamber.

The output of the magnetron is 10 kW at 2.45 GHz. A stabilized power supply maintains a constant power output from the magnetron with input voltages varying 5% from 200 VAC (50/60 Hz, rms). The magnetron is water cooled, and a thermal switch limits the temperature rise. The duration of irradiation can be set from 0.01 to 1.99 sec in 0.01 sec units. Peak power is obtained within 0.01 sec.

The tuning system transmits a low energy 10 mW microwave signal which is sufficiently low in power so that there is no perceptible increase in the temperature of the brain. The system consists of a solid state oscillator with the signal transmitted from a coaxial antenna into the wave guide. The energy reflected from the head of the animal in the exposure chamber is monitored on a comparator meter. Tuning is accomplished by minimizing the reflected power through use of a stub tuner.

Two different size wave guide access tubes are used: one for positioning the rat and the other for positioning the mouse in the microwave field. These tubes are set on the wave guide at right angles to each other.
When the distribution consists of the TE\textsubscript{10} wave, the maximum energy of the electric field (E-field, E) can be obtained at the position of 1/4 wave length (\(\lambda\)) and 3/4 (\(\lambda\)) from the electric short end. The direction of the electric field is perpendicular to magnetic field (H-field, H) and is changed at the speed of 2.45 \times 10^9 times per second. The tubes are located 1/2\(\lambda\) from the electric short end, so the animal’s head is positioned in the magnetic field when the holders are inserted.

**Animal containers:** Three different sizes of containers were built to fit rats of 200, 300 and 400 g. These containers were designed to minimize the movement of the animal’s head position during MWR by incorporating a V-shaped cradle for the head. The rat container was designed so that the rat could bring both forelegs forward thus making it possible for the rat to stretch its head forward. The rat would not readily extend the head forward when the forelegs were pressed backward. The containers were designed as a dark tunnel with a light at the forward end. This was very successful because the rats attempted to crawl toward the light and in their effort placed their heads in the V-shaped cradle.

The shape of mouse container is slightly different from that of rat. The container consists of one cylinder with a truncated cone on one end for restricting the large movements of the mouse head.

**Measurement of temperatures of brain areas following microwave irradiation:** Rats weighing 250 to 420 g and 18 to 28 g mice
were used. Rats were irradiated at 9.0 kW for 0.8 sec and mice for 0.33 sec. The temperature of the brain was measured by inserting a needle containing a thermocouple in the tip. Temperatures of five portions in rat and mouse brain following irradiation are shown in Fig. 2. The exact placement of the needle tip in the brain was established by careful dissection of each brain following the temperature measurement. The time required for measuring the temperature of the five areas was 60 sec for the rat and 30 sec for the mouse.

**Thermography:** A thermoviewer, JTG-500 M (JEOL, Japan) was utilized for the visualization of heat distribution in the rat and mouse brain following microwave radiation. Rats and mice to be studied were sacrificed by carbon dioxide inhalation and then frozen in a −70°C cold room. The frozen head was then cut in either the sagittal, frontal and horizontal plane using a diamond edged saw. After the temperature of the brain returned to room temperature (27–28°C), the parts were reassembled with tape and placed in the exposure chamber. The rat’s brain was irradiated at 6 kW for 0.6 sec and the mouse brain was irradiated at 6 kW for 0.12 sec. After irradiation, one side of the truncated head was removed from the body and quickly set in front of the camera unit. Each frame of a thermal image with its temperature information was stored in a data memory and the image of the brain surface was displayed at a flicker-free rate on a cathode ray tube under the most optimum display conditions. Temperature resolution of the device was 0.05°C.

**Heat distribution using succinic dehydrogenase:** Rats were sacrificed by MWR at 9.0 kW for 0.4 or 0.8 sec, with rats sacrificed by decapitation serving as the control for enzyme activity. The brains were then removed from the skull and frozen in an acetone dry-ice mixture. They were then cut into 0.5 mm sections, and succinic dehydrogenase was assayed histochemically by the method of Nachlas et al. (33). The slice of tissue was immersed in a mixture of 7 ml of 0.1 M phosphate buffer, pH 7.4, 1 ml of 0.5% nitro blue tertzazolium (NBT) solution, 1 ml of 0.2 M sodium succinate, pH 7.4, and 0.2 ml of 0.02% phenazine methosulfate solution. After incubation at room temperature for 20 min in the dark, the areas of active enzyme were stained a rich blue color.

**Assay of acetylcholinesterase:** Forty rats were divided into 8 groups including one control group sacrificed by decapitation. The seven other groups of rats were sacrificed by microwave irradiation with a power of 9 kW for a time duration of 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 or 1.0 sec. Acetylcholinesterase activity was analyzed in individual whole brains by

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**Fig. 2.** Outline of the rodent brain indicating locations (a–e) in the brain where temperatures were measured following MWR. Temperatures were measured by inserting a needle containing a thermocouple in the tip into the lettered region. The recorded temperatures are noted in Table 1.
the Ellman method (34). Each brain was homogenized in 3 ml of 0.1 M phosphate buffer (pH 9.0) with a Potter-type homogenizer. After the homogenate was diluted 100 fold with the same buffer solution, 0.4 ml of diluted solution was transferred to another tube containing a mixture of 2.6 ml of the buffer solution, 100 µl of 5,5-dithio bis-2-nitro benzoic acid (DTNB) reagent and 20 µl of substrate solution. The DTNB reagent was prepared by dissolving 39.6 mg of 5,5-dithiobis-2-nitrobenzoic acid and 15 mg of sodium bicarbonate in a solution of 10 ml of 0.1 M phosphate buffer (pH 7.0). Acetylthiocholine iodide (0.075 M) was used as the substrate. Immediately after mixing the tissue with the substrate, the rate of color production was measured in a spectrophotometer at 412 mÅ.

**Assay of acetylcholine, catecholamines and 3,4-dihydroxyphenylacetic acid (DOPAC):** Twenty rats were divided into two groups: the control group was sacrificed by decapitation, and the other was sacrificed by microwave irradiation at a power of 9.0 kW for a time duration of 0.9 sec. The brain of each rat was then immediately removed from the skull and dissected into seven regions according to a method based on that of Glowinski and Iversen (35). The regions were the cerebral cortex, medulla-pons, hypothalamus, striatum, hippocampus, cerebellum and midbrain-thalamus. Acetylcholine was measured by a pyrolysis gas chromatograph-chemical ionization quadrupole mass spectrometer (JMS-QH 100 PGC/Cl/QMS) coupled with a basic JMA-200 computer system and a high-speed graphic output system which include a VP-55 video printer and multiple ion detection capabilities. The gas chromatograph was equipped with a glass column, 1.0 m×3 mm I.D., packed with 5% OV-101, 5% dodecylmethyl-ethylenetriamine succinamide on 80–100 mesh Gas-Chrom Q (Gasukuro Kogyo Inc., Japan). The carrier gas was helium for the combined GC-MS studies. The flow-rate was 30 ml/min. The temperature of the column was 110°C, the injection port was at 140°C, and ion source at 170°C. Details of pyrolysis conditions (36) and extraction procedures (37) have been previously reported.

Norepinephrine, dopamine, epinephrine and DOPAC were analyzed using high performance liquid chromatography with electrochemical detection (38). This system consisted of a model 112 Solvent Delivery System combined with an electrochemical detector (Bioanalytical System Inc., Model LC-3A), which was set at +0.6V versus the silver-silver chloride reference electrode. The detector was attenuated at 5 to 20 nA full scale, depending on the concentration of catecholamines. The working electrode was of glassy carbon. The chromatograph used a stainless steel column, 125 mm long×5.0 mm i.d. (Chemco Scientific Co., Ltd., Japan), packed with Chemcosorb C_{18} (particle size 3 µm); and the mobile phase used was 0.05 M citrate buffer at pH 4.8, containing 8% methanol and 0.09 mM sodium octyl sulfate.

**Electron microscopic observation of brain tissue following MWR:** Half the rats were sacrificed by MWR at 9.0 kW for 0.8 sec, and the other half were sacrificed by decapitation. The resulting mean temperature±S.D. in MWR sacrificed animals was 91.4°C±2.91.

Electron microscopic specimens were prepared by fixing full thickness pieces of tissue (0.5/0.5 mm) with 2.5% gluteraldehyde in Millanig buffer for 3 hr at 4°C and then postfixed with 2.0% osmium for 1 hr at 4°C. Tissue samples were then dehydrated in a graded series of alcohols and embedded in an Epon mixture. Examination of the specimens was performed with a model JEM-1200EX JEOL electron microscope (21).

**Results**

**Instrument:** The microwave system used has an integral tuning unit to allow adjustment of the instrument to the size and configuration of the tissue. The instrument operated at a delivered 9 kW of power measured by the temperature rise in a water filled dummy load. Peak power was obtained within 0.01 sec. The power distribution attained is shown in the following tissue studies.

**Animals:** The rats and mice readily entered the containers and seldom struggled while in the container. The time required for completion of the entry of the animal into the container followed by sacrifice and enzyme
denaturation seldom exceeded 15 sec.

Appearance of the rat brain following microwave irradiation in H-magnetic (H) and E-electric (E) field modes: The gross appearance of the brain following MWR in H-field and E-field is shown in Figs. 3 and 4. Most available and currently used microwave units utilize the E-field with a power output of 3–5 kW. This power was used to evaluate the heating effect in the H-field as compared with that of the E-field.

Comparison of the tissue appearance in dorsal, (Fig. 3A and 4A) ventral (Fig. 3B and 4B) and sagittal (Fig. 3C and 4C) views shows significant differences in uniformity of heating between the fields; the appearance of the tissue irradiated in the H-field mode was much more uniform than that of the tissue exposed to the primarily E-field mode. Rapid and uniform coagulation of blood is important for limiting contamination of compounds between blood and tissues. We did not specifically study this problem, but most analytical techniques for studying

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Fig. 3. Appearance of dorsal (A), ventral (B) and sagittal (C) aspects of the rat brain following MWR at 9 kW for 0.8 sec in the H-field.

Fig. 4. Appearance of dorsal (A), ventral (B) and sagittal (C) section of the rat brain following MWR at 5 kW for 1.5 sec in the E-field.
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brain tissue include the blood in the tissue analysis. If the blood content of a specific compound were of interest, it is relatively easy to isolate the pial vessels containing the coagulated blood. There are visible in the photographs several traces of burned patches and damaged tissue in the E-field identifying the non-uniformity of microwave energy absorption and dissipation. This tendency also is clear in the sagittal plane section of the brain.

Measurement of temperatures in brain areas following microwave irradiation: The regional temperatures in rats and mice brain shown in Fig. 2 were measured by the procedure described in the Materials and Methods Section, and the results are summarized in Table 1. Following irradiation at 9.0 kW for 0.8 sec for the rat and 9.0 kW for 0.33 sec for the mouse, the range of temperatures attained was 91.4 to 98.3°C for rats and 76.7 to 80.6°C for mice. The maximum difference in the temperatures in the brain following microwave irradiation was 6.9°C for rats and 3.9°C for mice.

In animals treated the same way, no significant differences in temperatures were found within each point "a" to "e" in rats and mice brain areas. The heat distribution to a certain extent centered in the areas midbrain to the pons in both the mouse and rat.

The average temperature difference between the rat, 94.0°C, and the mouse, 78.9°C, depends on the duration and power of the incident irradiation. The temperature difference in both mouse and rat brain from b to d was virtually the same value, although the temperature difference between the locations a and e in the rat brain was 4.5 times higher than that of the mouse.

Table 1. Temperature of rat and mouse brain areas (a to e)*1 following microwave irradiation at a power of 9 KW

| Brain Area | Mean temperature °C±S.D. |
|------------|--------------------------|
|            | Rat *2                  | Mouse *3               |
| a          | 91.6±1.26               | 77.6±1.48              |
| b          | 91.4±2.91               | 76.7±3.81              |
| c          | 93.7±2.60               | 80.3±2.62              |
| d          | 95.0±1.85               | 80.6±2.29              |
| e          | 98.3±1.63               | 79.1±2.73              |
| x (a–e)    | 94.0±2.14               | 78.9±2.69              |
| T°C difference (b–d) | 3.6  | 3.9                |
| T°C difference (a–e)  | 6.7  | 1.5                |

n=6. *1See Fig. 4 for anatomical locations of the areas a to e. *2Temperature measured within 60 sec after 800 msec irradiation. *3Temperature measured within 30 sec after 330 msec irradiation.

Heat distribution in rat and mouse brain by thermography: Heat distribution due to absorption of microwave energy was displayed and photographed as a color image of the cut surface of the rat and mouse brain. The photographs are shown in Fig. 5. In order to evaluate the effect of energy absorption and its conductivity in the tissue, it was critical to irradiate the brains with a low power and duration of microwave.

Therefore, rats were irradiated at 6 kW for 600 msec and mice at 6 kW power for 120 msec. In rats, the highest energy was centered in the middle of the plane and distributed to the periphery. The temperature differences recorded in rat's sagittal, frontal and horizontal sections were 4.6°C (40.9–36.3°C), 5.0°C (44.0–39.0°C) and 3.4°C (37.9–34.5°C), respectively. Temperature differences in rat brain by MWR were below 5.0°C under this experimental condition. The mark "Ar" (Fig. 5) in the sagittal, frontal and horizontal planes identifies artifacts from an edge of the material used for supporting the tissue.

The temperature difference of 5.0°C obtained with thermography compared
favorably with that of 6.7°C obtained by a thermometer in the previous section. In the mouse brain, the highest energy of microwave was also centered in the brain. The differences in temperatures in sagittal, frontal and horizontal planes were recorded as 1.5°C (28.5–27.0°C), 0.8°C (27.9–27.1°C) and 1.2°C (28.0–26.8°C), respectively.

The energy deposition was also shown to have approximately the same distribution pattern as occurs in the rat brain. The absorbed energy in the center was conducted to the peripheral areas in the shape of an expanding circle. The temperature difference of 1.5°C in mouse brain as measured by thermography exactly coincides with that measured by the thermocouple as shown in Table 1.

**Succinic dehydrogenase activity as a measure of heat distribution:** To obtain a qualitative evaluation of microwave energy, distribution of succinic dehydrogenase activity was examined following MWR. The enzyme is readily inactivated by heat, and the use of p-nitrophenyl substituted ditetrazole permits the cytochemical visualization of the sites of enzyme activity in 2–8 μm thick brain tissue slices under aerobic conditions, after a relatively short incubation period. The procedure was applied to decapitated brain tissue and brain tissue following microwave irradiation in order to visualize the effect of heating on inactivation of the enzyme. As shown in Fig. 6, the blue color showing a darker shade in the control tissue (A) was significantly reduced following irradiation at 9.0 kW for 0.4 sec (B) and the color entirely disappeared following microwave irradiation at 9.0 kW for 0.8 sec (C).
This indicates a complete inactivation of the enzyme. The average temperature in the midbrain following 0.4 sec radiation was 56.8±1.8°C and for 0.8 sec, it was 92.4±1.1°C as measured with the thermocouple.

The effect of microwave heating on cholinesterase activity: Cholinesterase activity in the rat brain was determined following various degrees of microwave heating in order to confirm the critical temperature for destroying the enzyme.

As shown in Fig. 7, 52.2% of cholinesterase activity was destroyed by 0.4 sec radiation at 9.0 kW power. In this case, the midbrain temperature was 56.8±1.8°C. Successively, 99.7% of the enzyme was inactivated by 0.6 sec radiation at the same power, for which the brain temperature was recorded as 70.1±3.7°C. This result is in accordance with those reported by Stavinoha et al. (2).

Effect of microwave irradiation on the concentration of acetylcholine, catecholamines and metabolites in the brain: The concentration of regional acetylcholine in rat brain was determined following MWR at 9.0 kW for 0.9 sec and compared with levels obtained from decapitated animals (Table 2). Regional levels of acetylcholine following MWR are in agreement with those of other reports (24, 30, 39-41). The highest concentration was found in the corpus striatum and the lowest in the cerebellum.

Significantly higher acetylcholine concentrations were noted after MWR in all areas except the cerebellum. In this experiment, brain tissue was homogenized within 10 min after MWR.

The levels of norepinephrine, epinephrine, dopamine and DOPAC in brain areas of the rat were assayed by an HPLC-ED system following microwave irradiation at 9.0 kW for 0.9 sec, and the results were compared with those following decapitation (Table 3).

The highest concentration of norepinephrine was found in the hypothalamus, and lesser amounts were found in the midbrain-thalamus, medulla-pons, hippocampus and corpus striatum. The lowest concentrations were present in the cortex and cerebellum. The highest level of DOPAC was found in the corpus striatum, where dopamine concentration was also the highest. The lowest concentration of DOPAC was found in the cerebellum.

A statistical decrease as compared to decapitation was evident in the DOPAC level in the corpus striatum, cortex, medulla-pons and midbrain-thalamus. The highest concentration of dopamine was in the corpus striatum where the largest dopaminergic tracts are located. A significant increase of
dopamine was found in all areas following MWR. Part of the variation seen in the significant differences between decapitated animals and microwave sacrificed animals may be due to the variable time interval that follows decapitation before enzyme inactivation occurs.

Very little information on epinephrine levels has been reported, and no significant difference was observed in their concentrations between the two methods of sacrifice in this experiment.

**Electron microscopic observation of brain tissue following microwave irradiation:** For the histochemical examination, sections of the corpus striatum, locus coeruleus, cortex and hypothalamus were prepared from rat brain after MWR, and the results were compared with those obtained from the decapitated tissue sample. Both light and electron microscopic examinations were performed to visualize the interface between the striatum and cortex, the shape of the nerve cell, the general tissue structure, and the preservation of the synapse and synaptic vesicles. Histochemical examination on tissue heated by a 5 kW device has been reported (21, 22).

In Fig. 8, electron micrographs of cortex sections following MWR and decapitation are presented. There was no significant structural destruction in sections prepared either way, although slight vacuolation was seen in the tissue following MWR. Synapses and synaptic vesicles were well defined in both sections. Mitochondria can be seen along
Table 2. Acetylcholine concentration in n mole/g±S.D. in rat brain regions following two sacrifice methods

| Brain region       | Decapitation | Microwave irradiation (9.0 kW–0.9 sec) |
|--------------------|--------------|----------------------------------------|
| Cerebellum         | 6.4±1.0      | 7.3±1.8                                |
| Medulla-pons       | 20.7±3.6     | 29.3±6.0**                            |
| Hypothalamus       | 23.6±3.2     | 38.6±5.1**                             |
| Corpus striatum    | 34.0±5.9     | 80.0±9.4**                             |
| Midbrain-thalamus  | 26.7±4.1     | 39.5±6.7**                             |
| Hippocampus        | 15.1±2.3     | 29.5±3.2**                             |
| Cortex             | 9.1±2.3      | 24.1±3.1**                             |

n=10. **Significantly different from the control, P<0.01

Table 3. Regional catecholamines and metabolite concentration in ng/g±S.D. in rat brain following sacrifice by decapitation or microwave irradiation at 9 kW for 900 msec

| Region          | NE Decapitation | MWR | EP Decapitation | MWR | DOPAC Decapitation | MWR | DA Decapitation | MWR |
|-----------------|-----------------|-----|-----------------|-----|--------------------|-----|-----------------|-----|
| Cerebellum      | 289±43          | 530±65**          | ND  | ND               |      | 15±9             | ND  | 12±3           | 26±7**          |
| Medulla-pons    | 529±51          | 589±69            | 8±3 | 11±3            |      | 42±6             | 25±12** | 45±8           | 55±6           |
| Hypothalamus    | 1229±161        | 1263±41           | 34±2 | 35±2            |      | 169±44           | 144±13 | 368±40         | 538±49**        |
| Corpus striatum | 388±82          | 429±64            | ND  | ND              |      | 2243±161         | 801±44** | 6291±465       | 7159±231**      |
| Midbrain-thalamus| 544±57          | 661±79**          | 5±3 | 9±1             |      | 74±7             | 55±4**  | 110±15         | 205±77**        |
| Hippocampus     | 502±76          | 578±69            | ND  | ND              |      | 54±20            | ND  | 24±6           | 94±15**         |
| Cortex          | 289±42          | 543±37**          | ND  | ND              |      | 304±61           | 121±18** | 268±51         | 589±85**        |

n=10. *P<0.05. **P<0.01. ND=Not detectable
with synaptic vesicles and the synaptic cleft in the tissue sections following decapitation sacrifice; however, the mitochondrial cleft following MWR was vague, suggesting denaturation of the enzymes.

**Discussion**

We have used the criteria presented in the introduction to evaluate the applicability and practicality of microwave heating to inactivate tissue enzymes at sacrifice. This procedure makes possible preservation of enzymically labile compounds. The wide distribution of power in the brain was accomplished by use of a tuned, predominantly magnetic field for brain exposure. This approach was initially reported in an application using a prototype instrument with mice (6) This original instrument was built in the laboratory and was not commercially available. Utilization of an integral tuning system consisting of a milliwatt transmitter and cathode ray tube for optimizing the power distribution in the head of the mouse was also used in this instrument. Neither feature has been here-tofore commercially available (6). Efficiency of the distribution of the microwave energy is optimized by the tuning system. The percent of reflected power can be estimated at 3–5 percent for mice and 2–3 percent for rats, a definite advantage over a fixed untuning instrument. Full power is attained in $1.5\pm0.2$ msec.

Studies using a thermocouple to measure the temperature of the irradiated rat brain show that a relatively even distribution of heat occurred. Irradiation for 800 msec increased the temperature of the rat brain to a maximum of 98.3°C, with a maximum difference of 6.9°C between areas. In the mice, the maximum temperature was 80.6°C, with a maximum difference of 3.9°C between areas. There was no significant difference in temperatures between individual rats with the same exposure to microwave heating. This was also true for the individual mice with the same exposure. This is a very important factor governing uniformity in results, and it markedly reduces the rejection of animals in a series that can occur because of the possibility of incomplete enzyme inactivation. Knowledge of the heat stability of labile compounds is essential if they are to be determined following microwave irradiation. Effects of temperature on the stability of acetylcholine, catecholamines and their metabolites were carefully determined and confirmed by preliminary tests (2, 23). Only negligible amounts were decomposed during MWR heating.

The widespread distribution of heat with the magnetic field is particularly apparent by
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thermographic visualization. Temperature differences in the rat brain were 6.7°C and in the mouse, 1.5°C. This is in contrast to the commonly used electric field distribution which concentrates the energy input into a relatively small area. Temperature measurements following use of the electric field mode will often show a 15°C or more temperature difference in central versus peripheral brain regions. This can be of use when studying a specific area of the brain that can be differentially heated, but is a damaging drawback in studying whole brain areas. In different sizes of animals treated in the same manner, only slight changes in temperature distribution were found. In very large animals, the olfactory lobes and the brain stem would tend to be a lower temperature than other parts of the brain. If, during rapid heating, the microwave energy is localized in one small area the amount of energy that will be required to pass through that area in order to heat the other areas of the brain will drive the temperature beyond 100°C. This will increase the pressure inside the brain and often cause displacement and severe damage to the brain structure.

The succinonic dehydrogenase activity was significantly decreased following 400 msec irradiation and absent following 800 msec irradiation. The activity of cholinesterase began diminishing at 300 to 400 msec of irradiation, that is, a temperature of 50 to 60°C, and it disappeared at 600 msec, at a temperature of 70°C. This corresponds closely to other recently reported data on temperature denaturation of cholinesterase (6). Thus 600 msec is an adequate exposure time for study of the central cholinergic neurotransmitter concentrations. Choline acetyltransferase is much more heat labile than cholinesterase and would be inactivated earlier, at a lower temperature.

The acetylcholine concentration in six of seven brain regions is significantly higher following microwave irradiation for 900 msec as compared to decapitation sacrifice. It is well known that decapitation drastically changes the state of acetylcholine in the brain since part of the acetylcholine is immediately susceptible to post-mortem destruction by cholinesterase. The rate of post-mortem decrease in some parts of the brain is known to be 1–5 nmole/g/min. The amount of the change following MWR as compared to decapitation will depend on the experimental conditions and the region of the brain (40). The mechanism of post-mortem changes and metabolism of acetylcholine following MWR have been studied by Stavinoha et al. (30) and Weintraub et al. (40). The cerebellum was the only area which showed no significant change. The cerebellum has the lowest synthesis rate for acetylcholine of the seven regions (42). The ratio of choline acetyltransferase to cholinesterase, CAT/CHE, is quite low, $3 \times 10^3$ in the cerebellum, as compared to areas such as the cerebral cortex with $12 \times 10^3$ and the striatum with $13 \times 10^3$ (43). Weintraub et al. (40) have reported a slight increase in acetylcholine concentration in the cerebellum in rats heated by microwave 5 min following decapitation. Distribution of acetylcholine in these brain regions was in accord with that of cholinesterase (44). The changes following sacrifice are complex and governed by many factors. The difference between the rapid inactivation values and those of decapitation depends upon the way the acetylcholine is handled and stored in the region. We did not do extensive tests in stress-related changes in the brain because the handling technique used for sacrifice appeared to be the least traumatic of the methods of sacrifice. The technique employed by Blank et al. (23) that is a comparison of decapitated, decapitated plus microwave heating, and microwave heating alone indicated no change in the catecholamines studied. Undoubtedly, as our methods improve, stress-related changes in the brain will be noted.

A relatively large number of studies on catecholamine concentrations in the brain have been done recently (45–57). Early studies on the effect of rapid microwave heat inactivation on the catecholamines in the brain indicated no change, a slight change, or brought up the possibility of diffusion or pressure artifacts (27, 29). More recent studies have indicated that changes do occur in the concentration of catecholamines in certain regions (23, 45, 58, 59). Comparison of data between laboratories in still difficult
because of variations in the method of analysis, sacrifice technique, time of heating and distribution of power. The results reported for the rat following 900 msec irradiation in this paper show that significant regional differences in norepinephrine, DOPAC and dopamine do occur when compared with decapitation sacrifice. The evaluation of stress to animals of being placed in the chamber was carefully performed by the method of Blank (23). No effect of pressure induced changes on levels of the assayed compounds was found between the two sacrifice procedures.

Examination by electron microscopy of the microwave heat fixed tissue was done to evaluate the possibility of structural damage caused by the heat exposure. It was concluded that the changes of compound levels were not the result of tissue disruption following rapid heating of the brain by irradiation. Histochemical fluorescence of brain catecholamines is diminished following microwave irradiation because there is a temperature-dependent decrease of formaldehyde indirect fluorescence in brain tissue that has been heated. Use of glyoxylic acid in the procedure resulted in an increase in fluorescence to a level comparable to unheated tissue (21, 22). Evaluation of microwave irradiation using the glyoxylic acid method demonstrated that proper microwave heating preserves the integrity of the tissue with no discernable diffusion of the catecholamines.

The use of this instrument to rapidly sacrifice and denature enzymes in the central nervous system of small animals improves the accuracy and precision of the data since the degradation and synthesis of compounds can be stopped during a precisely known time interval. The technique is also relatively atraumatic, with unconsciousness occurring within approximately the first 10 percent of the exposure period. The use of rapid microwave sacrifice should result in more comparable concentrations of enzymatically labile compounds in the brain being reported between laboratories. This improved accuracy should lessen the need for so many laboratories to perform repetitive experiments.

In summary: The ability of a microwave enzyme inactivating instrument to heat and inactivate enzymes in the brain without causing tissue disruption was studied. Following this, the effect of rapid inactivation on levels of labile compounds in regions of the brain was evaluated. Except for epinephrine, acetylcholine, norepinephrine, DOPAC and dopamine showed changes in concentration in regions of the brain following rapid inactivation when compared to a slower method of sacrifice.

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