A Microwave Applicator for *In Vivo* Rapid Inactivation of Enzymes in the Central Nervous System

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***Abstract*—The paper describes modifications of microwave techniques for *in vivo* rapid inactivation of brain enzymes. These modified techniques offer greater rapidity and homogeneity of inactivation. The microwave-treated brain remains suitable for regional dissection.**

INTRODUCTION

Cyclic adenosine monophosphate (cyclic AMP) has been established as an intracellular mediator of the action of a number of hormones [1]–[3], and may play an important role in the function of the central nervous system [4], [5]. Determination of the concentration of cyclic AMP in various regions of the brain therefore provides an important tool in neurochemical research, e.g., in evaluation of the effects of hormones or drugs in the central nervous system. In order to reliably determine the distribution of cyclic AMP, it is necessary, in the process of sacrificing the animal, to rapidly denature (inactivate) the enzymes that both produce and degrade cyclic AMP, i.e., adenylate cyclase (AC) and phosphodiesterase (PDE), respectively. These enzymes, if left active for even a few seconds, will produce artifactual increases in the levels of cyclic AMP that do not reflect actual endogenous concentrations prior to sacrifice [6]. This rapid anoxic activity of enzymes seen with cyclic AMP during the postmortem period also occurs with many other important metabolites in the brain [7], [8].

Conventional methods of sacrifice use liquid nitrogen to freeze the tissue and thereby stop enzymatic activity. Most freezing methods using immersion into liquid nitrogen inactivate enzymes nonuniformly throughout the brain, and may require up to 90 s for total inactivation [9]. In addition, freezing methods do not permit dissection at room temperature because any postmortem thawing of tissue allows enzymatic activity to resume. The more rapid freezing methods such as freeze-blowing [10] have decreased the time of inactivation considerably, but do not permit regional dissection since there is a loss of anatomical features.

Microwave heating provides a promising approach that is based upon the principle that cyclic AMP is a relatively heat-stable substance [11], while the enzymes (AC and PDE) involved in its metabolism are heat labile and denature irreversibly at temperatures in the range of 65–90°C. Using sufficiently high microwave power, inactivation of enzymes can be achieved with exposure times on the order of 1–2 s or less, permitting subsequent regional dissection of the brain at room temperature. An important requirement in the design of a microwave inactivator, however, is the need for uniform heating of brain regions. This paper addresses one such attempt to design an applicator that would both couple the animal to the field more efficiently and improve upon the uniformity of the heating pattern.

METHODS

Early microwave inactivation procedures for the rat exposed the whole body within an oven cavity at a frequency of 2450 MHz. In our laboratory, the exposure time required for adequate inactivation of enzymes in carefully measured high-intensity regions of the oven ranged from 35–50 s at 2000-W forward power and 250 W reflected. The time required was dependent upon the size of the animal and varied with the field intensity obtained in various regions within the oven cavity. Subsequent reports in the literature demonstrated persistent anoxic artifacts at these times of exposure and the necessity for increasing the rapidity of the inactivation process [12]. In order to significantly increase the efficiency of the microwave inactivation of the brain, microwave exposure was restricted to the animal's head.

The first design, as shown in Fig. 1, consisted of inserting the animal's head through a hole in the broad face of a WR 430 waveguide. In an effort to concentrate the field at the rat head, we placed, on both narrow walls of the waveguide, aluminum shims which tapered to a maximum height in the region of the rat head, as noted in Fig. 2. Although inactivation times required decreased by 86 percent, to 5 s, there was variability of heating portions of the brain because of both the nonuniformity of the field being generated in the waveguide chamber and the mobility of the rat while in the field. The applicator design was modified to restrain the animal's head with a clamping device, eliminating any motion within the field. Studies demonstrated an improved reproducibility of results from animal to animal, but the heating pattern of the brain revealed a significant gradient.

At this point it became quite evident that not only was rapid inactivation required, but as one became increasingly interested in multiple discrete regions of the brain, a requirement of homogeneity of the inactivation process throughout the brain became most essential. In addition, the stress of vigorous immobilization of the animal was considered to be a possible confounding variable. Our laboratory has attempted to address both of these factors in the design and development of the microwave apparatus described as follows.

The arrangement used was a WR 430 waveguide test cell driven by a Varian PPS 2.5 generator and terminated in a short-circuiting

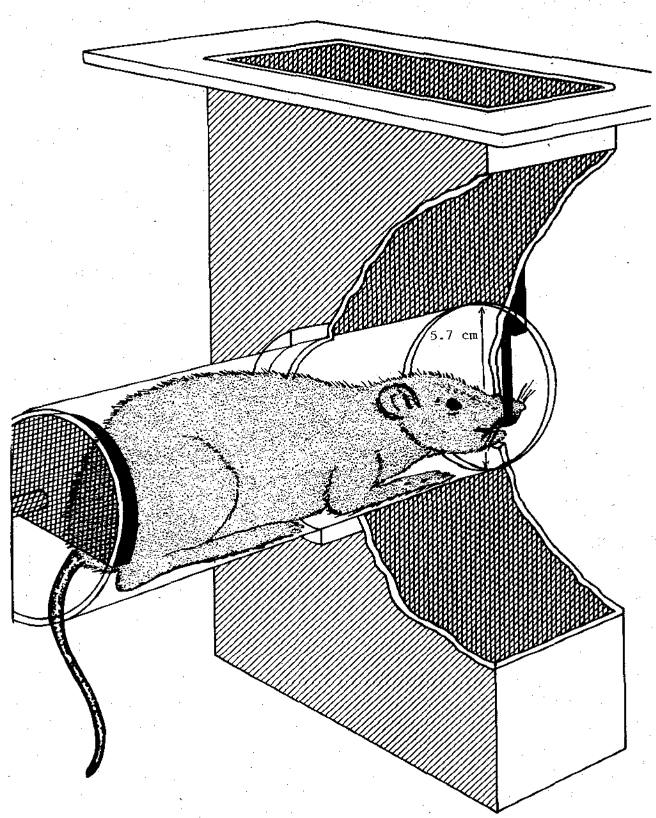

Fig. 1. Broad-face microwave applicator. Animal is placed in a Plexiglas tube which is inserted into the waveguide chamber.

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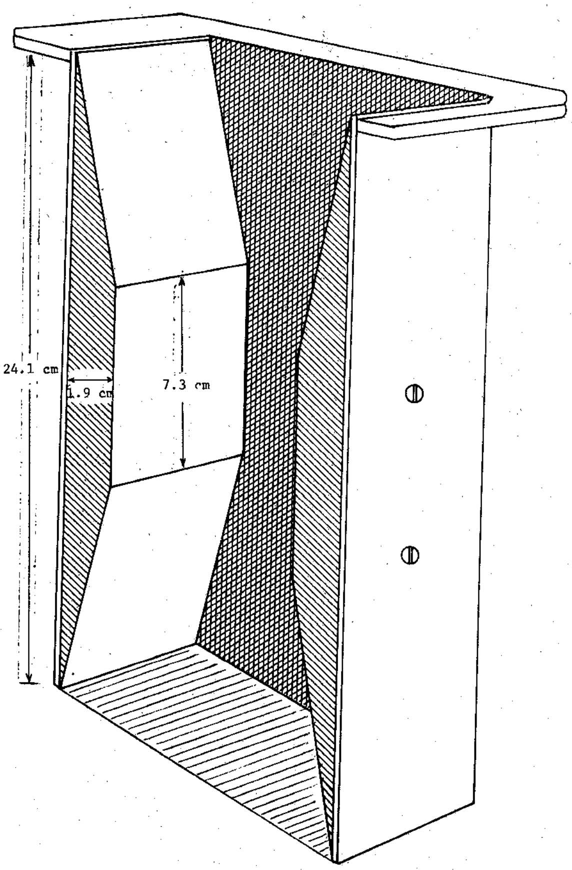

Fig. 2. Design modification of the waveguide chamber of the broad-face microwave applicator.

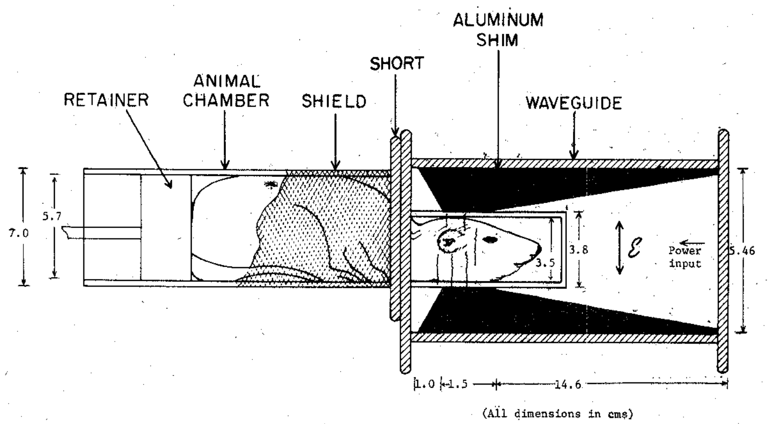

Fig. 3. Shorting endplate microwave applicator.

endplate with a central 3.8-cm-diam hole. The 3.5 kW of microwave power was matched to the complex load (the rat's head) by a double screw tuner. The hole diameter used was the minimum so as to allow the protrusion of only the rat head into the waveguide (Fig. 3). In addition to reducing the hole diameter, the choice of its location in the low-electric-field region at the shorting plate rather than at the high-field region at the broad walls of the waveguide helped to maintain the mode pattern and, hence, the symmetry of fields in the waveguide. Also, the leakage of power out of the waveguide was minimized because of the new hole location. The residual leakage from the hole in the shorting endplate was further reduced by wrapping copper screening around the 7.0-cm-OD Plexiglas tube used to hold the rat body. While allowing the visualization of the rat positioning, the screen acts as a cylindrical waveguide with the lowest TE_{11} mode cutoff frequency of approximately 2.5 GHz. The efficacy of shielding was confirmed by observing a power density of no more than 5 mW/cm^2 at a distance of 10 cm from the termination during 3500-W power exposures. The rat brain, of dimensions on the order of 2.5 $\times$ 1.8 $\times$ 1.0 cm, occupied a location centered in the maximum field region at a distance of $\lambda_g/4$ away from the short at the microwave frequency of 2450 MHz. The rat body was located in a 5.7-cm-ID tube external to the waveguide with the rat head in an offset 3.5-cm-ID tube protruding into the waveguide.

To concentrate the microwave power into the rat head and to obtain the uniformity of field distribution over the 2.5-cm longitudinal extent of the rat brain, the shorter dimension of the waveguide was gradually reduced (Fig. 4) to 3.8 cm by using two identical, tapered, aluminum plates attached to the broad faces of the waveguide. The plates tapered down over a length of 14.6 cm (to

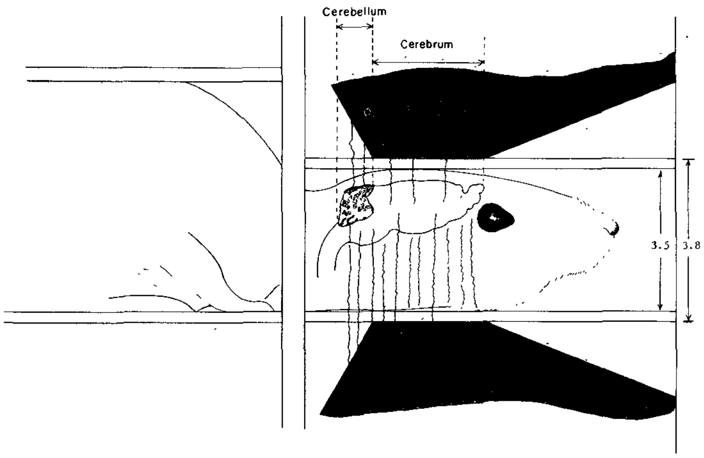

Fig. 4. Design modification of the waveguide chamber of the shorting endplate microwave applicator.

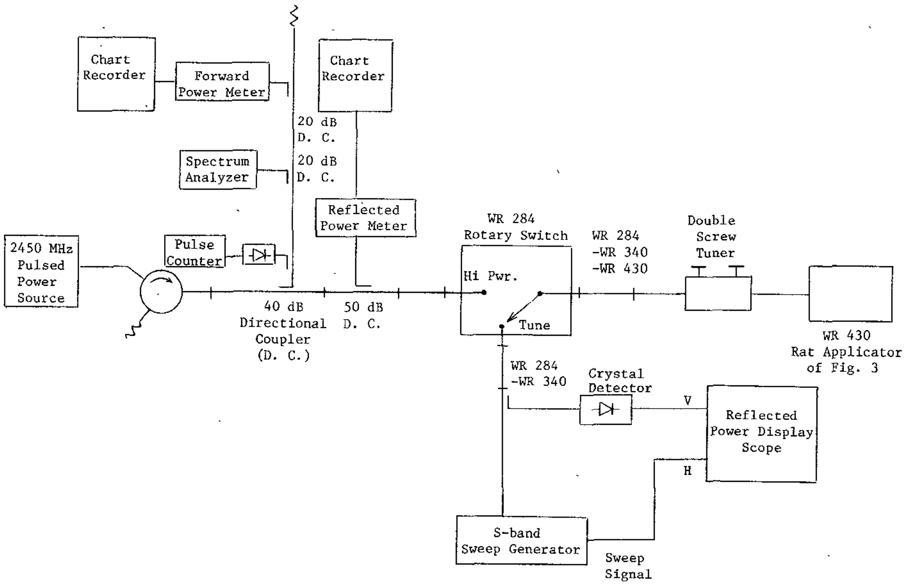

Fig. 5. Block diagram of the enzyme inactivator.

minimize incident power reflection) to a flat region of 1.5-cm length, and then abruptly returned to full waveguide height over an axial length of 1.0 cm. This produced an increase of fields away from the shorting endplate to compensate for the observed dielectric load of the rat head, which had otherwise resulted in a marked concentration of fields close to the short of the waveguide. Without this modification there was disproportionately greater heating of the cerebellum of the rat brain.

The block diagram of the microwave enzyme inactivator is shown in Fig. 5. The power is matched to the rat head by a double screw tuner, giving a fairly broad-band match as shown in Fig. 6. A low-Q match is essential since heating after a few RF pulses does result in altered dielectric properties of the load which, for narrow band matches, produce considerable reflection for subsequent pulses. Precise monitoring of the time duration of applied power is done by means of a counter that counts the number of microwave pulses. In order to accurately determine the applied microwave energy, a recording of the incident and reflected powers is obtained for each of the animals inactivated by the system.

Inactivation of brain regions was evaluated [13] and was shown to be more uniform using the construction outlined. The exposure time necessary for a 325-g rat was 2.8 s, with the animal becoming unconscious within fractions of a second upon application of microwave power [14]. The uniformity of fields along the width

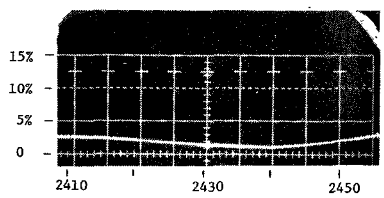

Fig. 6. Reflected power as a function of frequency for a double-screw-tuned rat applicator.

of the waveguide (from ear to ear of the animal) is obtained because of the symmetrical dielectric loading due to the rat head in that dimension, and the consequent concentration of power therein. Improved uniformity of heating of the brain was achieved in the rostral to caudal dimension (cerebrum to cerebellum) due to the tapered aluminum shims. In the vertical plane, however, a slight gradient (ventral greater than dorsal) was observed. This is currently ascribed to the overheating of the abundant muscle (because of its higher electrical conductivity) on the ventral side of the brain. The Plexiglas cylinder used to hold the rat in this study did not prevent rotation of the head in unanesthetized animals. Rotation of greater than 30° caused additional perturbation of the field and contributed to limited reproducibility from animal to animal. Further work is currently in progress to alleviate this difficulty.

CONCLUSIONS

The microwave applicators described herein have resulted in the simultaneous sacrifice and rapid inactivation of brain enzymes in the rat. Present results demonstrate control levels of cyclic AMP to be approximately 0.6-pmole/mg wet weight in the cerebellum. This is indicative of both the very rapid inactivation of the brain enzymes involved and the prevention of postmortem increase associated with more conventional methods of sacrifice. We have been able to measure levels of two cyclic nucleotides, cyclic AMP and cyclic GMP, in 13 distinct regions of the brain: cerebellum, brainstem, midbrain, substantia nigra, thalamus, hypothalamus, hippocampus, amygdala-pyriform cortex, septal nuclei, nucleus accumbens, olfactory tubercle, striatum, and cortex. Applicability of the technique to many putative central nervous system transmitters is being investigated in our laboratory [15].

Users of high-power microwave inactivation systems should be aware of the factors affecting the uniformity and reliability outlined here. We are continuing attempts to modify our microwave parameters to accomodate an increased mobility of the rat with improved uniformity and speed of inactivation.

ACKNOWLEDGMENT

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Letters

Dynamic Microwave Frequency Division Characteristics of Coplanar Transferred-Electron Devices in a Resistive Circuit

C. I. HUANG, J. TSUI, R. T. KEMERLEY, AND G. L. McCOY

Abstract—It is shown that dynamic microwave frequency division (divide-by-K) can be achieved by employing a transferred-electron device (TED) in a resistive circuit. The absolute bandwidth over which the input signal will be divided by a particular integer K and the maximum output frequency is the device transit time frequency. The percentage bandwidth is $200/(2K-1)$ percent. With two-terminal TED's, divide-by-K ($K = 2, 3, 4, 5$) was demonstrated with substantial bandwidth.

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INTRODUCTION

The use of transferred-electron devices (TED's) to perform dynamic microwave frequency division has been demonstrated by Upadhyayula and Narayan [1]. In their work, two-terminal sandwich-type GaAs TED's were tested in a coaxial cavity circuit. About 10-percent input bandwidth was reported at X band. The bandwidth capacity of the TED frequency divider has not been fully explored.

It is the purpose of this letter to derive the ideal frequency response and maximum bandwidth characteristics of an ideal TED in a resistive circuit. Experimental results are also included to substantiate the derivation.

SIMPLIFIED THEORY

It is well known that in the high-field-domain-mode GaAs TED's, a domain in transit must be quenched at the anode before a second domain can be nucleated [2]. Using this unique TED characteristic, frequency division can be easily achieved.