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# Brain hyperthermia alters local cerebral glucose utilization: a comparison of hyperthermic agents

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Microwaves have been proposed to alter neural functioning through both thermal and non-thermal mechanisms. We attempted to determine if local cerebral glucose utilization (LCGU) depends on the type of hyperthermic agent employed. We exposed the heads of rats to two different hyperthermic agents (5·6 GHz microwave exposure or exposure to hot/moist air) to create a 2°C rise in midbrain temperature. Other rats were sham exposed and remained normothermic. The 2-Deoxy-D-glucose (2DG) autoradiographic method was then used to determine LCGU during a 45-min period of stable hyperthermia. Hyperthermia (created by either hyperthermic agent) caused a general rise in brain glucose utilization. Hot-air exposed rats showed significantly higher LCGUs than microwaved rats in portions of the motor cortex, hypothalamus, lateral lemniscus and the substantia nigra (reticulata). Microwave exposure did not produce significantly higher levels of LCGU (compared to hot-air exposed hyperthermic controls) in any of the 47 brain areas sampled. A time analysis of lateral hypothalamic (LH) temperature during these different heating procedures revealed that microwave exposure produced a more-rapid rise in temperature than did hot/moist air. Thus, we wondered if the nuclei-specific differences in LCGU could be explained by localized differences in rate of brain heating during the two hyperthermic treatments. In a second study we carefully matched both the rate of lateral hypothalamic temperature rise and the peak temperatures achieved by our two hyperthermic methods and again measured LH LCGUs. We found that this precise matching eliminated the difference in hypothalamic LCGU previously observed following microwave or hot-air exposure. These data suggest that hyperthermia causes a general rise in brain metabolism and that (as long as steady state and rate of local brain temperature increase are well matched) microwave and hot-air induced hyperthermia produce similar changes in LCGU.

*Key words:* Brain, hyperthermia, microwave radiation, metabolism, local cerebral glucose utilization.

## 1. Introduction

Microwaves have been proposed to alter neural functioning through both thermal and non-thermal mechanisms (for reviews see Michaelson and Lin 1987, Merritt 1994). Here, we assessed neural function in rats by measuring local cerebral glucose utilization (LCGU) in an attempt to see if microwave exposure would produce metabolic effects similar to those observed following hot-air exposure.

Relative to other forms of hyperthermia (e.g. fever, those induced with external convection heating, etc.) microwave-induced hyperthermia's effects on brain metabolism have not been thoroughly investigated. However, Ho and Edwards (1977a,b),

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measured oxygen consumption in mice irradiated with 2·45 GHz in a waveguide exposure system. They found that the animals exposed to microwaves at a Specific Absorption Rate (SAR) $\geq$ 10·4 W/kg decreased their metabolic rate. Normal metabolic activity was resumed following cessation of exposure. These results suggest that the mouse compensates for the hyperthermia produced by a large dose of microwaves by adjusting its metabolic rate to offset the thermal load. Similar data have been collected in primates (for review see Adair 1987).

Other investigators have derived subcellular indicators of brain metabolism following exposure to microwave radiation. Sanders *et al.* (1980, 1984, 1985) Sanders and Joines (1984) have reported that exposure to 200–2450 MHz (producing brain SARs of 0·18–14·72 W/kg) inhibit mitochondrial electron transport chain function which results in decreased levels of brain adenosine triphosphate and creatine phosphate. These results are interpreted, by the authors, to reflect radiogenic decreases in brain energy metabolism. However, indirect effects due to changes in cerebral blood flow could not be ruled out.

More recently, metabolic measurements in brain have been refined through the development of an autoradiographic method that uses [$^{14}$C]-2-Deoxy-D-glucose (2DG) as a marker. Glucose is the primary energy source for brain tissue (Sokoloff 1984). For this reason recent investigations have concentrated on the measurement of cerebral glucose utilization as an indicator of both normal brain functioning and CNS damage (Sokoloff 1984). The 2DG technique has a tremendous sampling advantage over other methods in that it allows for simultaneous determination of LCGU and associated functional activity in all macroscopic structures of the brain.

The 2DG autoradiographic method has been used to measure the effects of environmentally induced temperature change on brain activity. McCulloch *et al.* (1982) reported a significant relationship between body temperature and the rate of brain glucose utilized. However, the magnitude of temperature-related alterations in glucose use showed considerable regional heterogeneity. In hyperthermic rats (rectal temperatures = 40·2 $\pm$ 0·3°C), significant increases in glucose utilization were observed in only 16 of the 38 areas examined (e.g. hypothalamus, hippocampus), whereas in a number of other major areas (e.g. cerebral cortex, thalamus), glucose utilization was minimally altered with hyperthermia. In a study focused on a more-limited number of brain areas, Murakami and Morimoto (1982) reported activation of hypothalamic metabolism and little change in substantia nigra, and other brain stem nuclei, during warming of the preoptic area. This group also studied the effects of fever ($>$ 2°C) on 2DG uptake into the brain. Again they reported increase metabolic activity in the hypothalamus but no change (or decreases in metabolism) in cortex, and limbic nuclei (Morimoto *et al.* 1986).

The above literature suggests that hyperthermia may produce either increases or decreases in brain metabolism. Apparently, the specific response observed may depend on a number of factors including the degree of heating, the portion of brain tissue sampled and the timing of the temperature measurement relative to the hyperthermic episode.

The specific means by which microwave radiation alters neural functioning and metabolism is uncertain. While the weight of data seems to point to a purely thermal effect of the microwave exposures (see Klaunberg *et al.* 1995), some have suggested 'non-thermal' mechanisms as well (for review see Michaelson and Lin 1987, Merritt 1994). In the current study, we used the 2DG method to measure how microwave-induced hyperthermia alters LCGU and then compared these data to normothermic

controls and a control group made hyperthermic via hot-air exposure. In an initial study, brain temperature matching was performed by using data obtained from midbrain probes. Later, we more-precisely characterized the temperature changes in specific brain nuclei (e.g. lateral hypothalamus and dorsomedial caudate) and matched both asymptote temperatures and rates of temperature rise in these areas. The data reveal that local brain metabolism is similar in microwave and hot-air exposed rats as long as the hyperthermia treatments are well matched for both asymptote temperature and rate of temperature rise.

## 2. Materials and methods

### 2.1. *Subjects*

We used male rats (weighing 332·5 ± 15·6 g) of the Sprague-Dawley CD-VAF/Plus strain obtained from Charles River (Portage, MI) colonies. The animals were individually housed (within an AAALAC accredited vivarium) in plastic 'shoe box' cages (44·45 cm long × 21·59 cm wide × 20·32 cm high) with rodent chow (Purina #5001) and water available *ad libitum*. Home cage temperature was maintained at 23–26°C under a 12/12 h light/dark cycle (lights on at 0600 h).

### 2.2. *Thermal controls and microwave dosimetry*

Following the method described by Burr *et al.* (1980a), we used hot/moist air to create a cephalic hyperthermia comparable to that observed during our microwave-exposure procedure. Briefly, our system consisted of closed hot and cold water baths from which we directed either hot/moist or cold air into a chamber (11 cm long × 10 cm wide × 8·5 cm high) containing only the head and anterior portion of the neck of our experimental subject. Air flow was computer controlled and the resulting hyperthermia was based on user-specified parameters of (1) desired rate of temperature rise and (2) desired temperature asymptote. The computer received continual feedback from an *in vivo* temperature probe and responded to the desired exposure parameters by rapidly raising or lowering hot or cold air flow. Humidity was maintained at 90–96% within the hot/moist air chamber.

Our microwave exposure system also provided computer control over cephalic temperatures. A 5·6 GHz Cober transmitter (Model 1326C-4 Series 2743) was used to provide microwave energy to the head and portions of the anterior neck at a localised, time averaged, Specific Absorption Rate (SAR) of 11·72 W/kg (Durney *et al.* 1986). Our computer controlled power by varying the pulse rate and thereby maintaining temperatures at user-specified levels using feedback from an *in vivo* temperature probe. Microwave pulse rate and width was achieved with an external TTL-compatible variable pulse rate generator (Questec Corp.) that pulse-modulated the microwave transmitter.

### 2.3. *Brain temperature measurements and pilot studies*

In order to determine the comparability of the brain temperature changes produced by our hot/air and microwave exposures, we performed a series of pilot studies. These studies also allowed us to specify the relationship between tympanic membrane temperature and brain temperature. Determining this relationship was critical since tympanic membrane temperature would be used to estimate brain temperatures during the 2DG experiments. Brain temperature probes were never used during the 2DG studies since these probes have been shown to disrupt cerebral

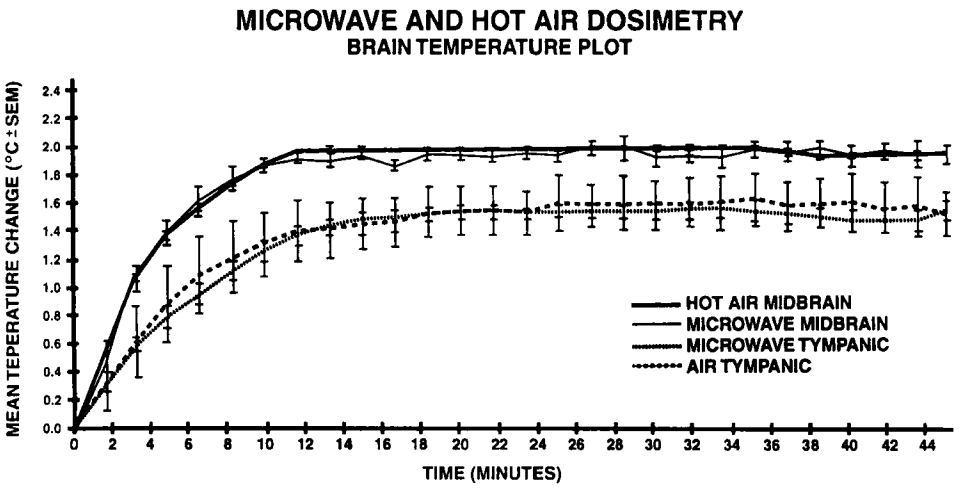


Figure 1. The relationship between rat tympanic temperatures and midbrain temperatures during exposure to hot/moist air or 5·6 GHz microwave radiaiton. Using these results from Pilot Study Number 1, the authors were later able to monitor tympanic temperatures and estimate brain temperatures while 2DG-injected rats were being exposed to one of these hyperthermic agents.

blood flow (CBF) (Verhaegen *et al.* 1992) and would presumably disrupt the LCGU measurements as well.

In an initial pilot study, rats ($N = 12$) were anaesthetized with ketamine HCl (50 mg/kg, i.p.) and Acepromazine (10 mg/kg, i.p.). Atropine sulfate (0·04 mg/kg, i.p.) was administered as a pre-anaesthetic agent in order to reduce salivation. Evidence suggests that ketamine has less effect on thermoregulatory responses than other anaesthetics (Hunter *et al.* 1981). However, ketamine has been shown to produce a marked heterogeneity in the pattern of LCGU values in the rat brain (for review see Crosby *et al.* 1982, Oguchi *et al.* 1982, Cavazzuti *et al.* 1987). In the current series of studies, both hyperthermic and normothermic animals were anaesthetized. Therefore, the metabolic effects of microwave and hot-air induced hyperthermia were superimposed on top of those induced by ketamine or other drugs given as part of the anaesthetic regimen.

A midsagittal incision was made in the scalp of the anaesthetized animal and a bilateral set of two holes (one per side) were drilled in the skull (−2·8 mm behind Bregma; ± 1·0 mm lateral from the midline). Using a stereotaxic instrument, plastic intracranial cannula (0·9 mm diameter) were placed in the holes and fastened there with plastic skull screws and Cranioplastic℗ cement. Microwave-compatible, non-metallic, temperature probes (BSD #21-10104-002) were later inserted through each hole and into the brain to a depth of 5 mm. This placement located the tip of the temperature probes in the midbrain dorsomedial thalamus (all temperature probe locations were verified histologically at the end of the study; Paxinos and Watson 1982). The heads of the anaesthetized rats were then exposed to either hot/moist air or microwave radiation as specified above. Probe placement in the brain can transiently reduce CBF and cause spreading depression. However, our measurements were made in a time period after implantation ($\geq$ 60 min) when spreading depression should have recovered significantly (Verhaegen 1992).

These preliminary measurements provided the following conclusions: (1) similar rates of midbrain temperature rise and asymptotes could be achieved through either hot/moist air exposure ($N = 6$) or microwave irradiation ($N = 6$) of the rat head (Figure 1); (2) using either of the hyperthermic techniques, we could achieve a $2{\cdot}0 \pm 0{\cdot}1$°C (Mean ± Standard error of the mean: SEM) rise in midbrain temperature in $12{\cdot}0 \pm 0{\cdot}2$ min. This 2°C midbrain temperature increase could be maintained for $\geq 45$ min; (3) tympanic temperatures were $0{\cdot}4 \pm 0{\cdot}1$°C lower than midbrain temperatures and the two measurements were highly correlated [$r(271) = 0{\cdot}97, p < 0{\cdot}01$]. These data allowed us, in future studies, to control midbrain temperatures by recording tympanic temperature and adjusting either microwave irradiation or hot/moist air exposure of the rat head during our 2DG experiments (see below).

Microwave -induced hot spots have been reported in a few brain areas (Ward *et al.* 1986) and have been explored theoretically (Burr *et al.* 1980b). We sought, therefore, to determine the asymptote temperatures and rates of temperature rise in selected brain nuclei during microwave and hot-air exposures outlined above. Further, if the two methods of producing midbrain hyperthermia did not result in a good match at the level of specific nuclei, we sought to develop procedures that would insure precise matching at the local level.

Using procedures similar to those employed in Pilot Study Number 1 (where midbrain temperatures were matched during hot air- or microwave-induced hyperthermia) we now measured local brain temperatures in the lateral hypothalamus (LH; a brain area where differences in microwave and hot-air induced LCGUs were observed—see data below) and dorsal medial caudate nucleus (DMC; where LCGUs were not significantly different during either microwave or hot/moist air exposures) (Table 1). In this second pilot study anaesthetized rats ($N = 10$; see Pilot Study 1 for anaesthesia regimen) were implanted with intracranial catheters that later held temperature probes (0·9 mm diameter) aimed at both the LH ($N = 10$; Stereotaxic Coordinates: −3·8 mm behind Bregma; ±1·6 mm lateral to midline; 9·0 mm skull depth) and the DMC ($N = 7$; 0·2 mm Anterior to Bregma; ±1·5 mm lateral to midline; 5·0 mm skull depth) (Paxinos and Watson 1982).

We found that, by monitoring tympanic temperature (reflecting a $1{\cdot}5 \pm 0{\cdot}1$°C rise in approximately 9 min = 0·2°C/min) we could control steady-state hyperthermic LH (Figure 2) and DMC (Figure 3) temperatures and keep them well matched between microwave and hot/moist air exposures [Mean ± SEM temperature increases: $1{\cdot}9 \pm 0{\cdot}2$°C for microwave-exposed LH, $N = 5$; $1{\cdot}8 \pm 0{\cdot}1$°C for Air-exposed LH, $N = 5$; $2{\cdot}5 \pm 0{\cdot}2$°C for microwave-exposed DMC, $N = 5$; $2{\cdot}3 \pm 0{\cdot}2$°C for Air-exposed DMC, $N = 5$]. This matching of plateau temperatures using our two hyperthermic methods was comparable to that observed when we measured midbrain temperatures during Pilot Study Number 1.

However, in Pilot Study Number 2, *rates* of *localized* brain temperature increase were not well matched between microwave and hot/moist air exposures (Figures 2 and 3). A repeated measures ANOVA compared the LH temperature measures recorded every 30 s during the initial 5 min of either the hot-air or microwave exposures. This analysis revealed that microwave exposure heated the LH faster than did hot-air exposure [$F(1, 9) = 5{\cdot}30$, $p = 0{\cdot}05$]. Specifically, the Mean ± SEM rate of temperature rise was $0{\cdot}4 \pm 0{\cdot}1$°C/min in the LH/microwave rats and $0{\cdot}3 \pm 0{\cdot}3$°C/min in the LH/ hot air rats. Similarly, the mean rate of temperature rise was $0{\cdot}6 \pm 0.1$°C/min in the caudate/microwave-exposed rats and $0{\cdot}4 \pm 0{\cdot}04$°C/min in the caudate/hot air-exposed rats. Thus, despite the good matching of

n ( ± SEM) local cerebral glucose utilization ($\mu$M/100g/min) during sham exposure (normothermia), microwave-induced
hot-moist air-induced hyperthermia.

| Brain area† | Sham | Microwave | Hot air |
|---|---|---|---|
| ucleus (ACb), | 170·46 ± 18·37 | 215·76 ± 24·63 | 227·18 ± 21·72 |
| ucleus, Basolateral (BL) | 162·73 ± 15·58 | 213·43 ± 26·71 | 240·87 ± 26·76 |
| ucleus, Basomedial (BM) | 148·51 ± 13·88 | 196·95 ± 32·55 | 235·95 ± 37·53 |
| ucleus, Lateral (La) | 162·41 ± 13·8 | 203·38 ± 31·71 | 240·67 ± 28·24 |
| ıen (CPu) | 168·73 ± 18·11 | 197·52 ± 18·63 | 226·64 ± 19·55 |
| CG) | 132·06 ± 17·66 | 133·91 ± 20·89 | 198·26 ± 32·36 |
| mi | 112·04 ± 12·39 | 88·16 ± 17·82 | 138·9 ± 25·21 |
| c | 132·97 ± 17·57 | 103·21 ± 18·12 | 156·99 ± 24·32 |
| ex, Anterior (ACg) | 218·24 ± 21·66 | 290·5 ± 21·46 | 313·87 ± 28·82 |
| ry (TeAud) | 196·25 ± 23·87 | 249·85 ± 25·44 | 235·32 ± 28·69 |
| ate, Posterior (PCg) | 241·51 ± 24·27 | 265·98 ± 27·65 | 311·51 ± 32·17 |
| l (Fr) | 161·47 ± 15·78 | 211·94 ± 23·2 | 229·78 ± 21·51 |
| parietal, Motor F(FrPaM) | 152·34 ± 14·78 | 190·69 ± 21·85 | 245·02 ± 26·1 |
| parietal Posterior, Motor(FrPaM) | 189·91 ± 23·61 | 230·42 ± 26·56 | 279·51 ± 24·3 |
| parietal, Posterior, SS (FrPaSS) | 170·36 ± 19·32 | 197·98 ± 24·81 | 278·83 ± 27·8 |
| parietal, SS (FrPaSS) | 136·44 ± 15·93 | 191·88 ± 11·35 | 225·51 ± 24·47 |
| | 223·24 ± 27·37 | 248·97 ± 29·88 | 259·03 ± 23·04 |
| cleus, Medial (MG) | 133·9 ± 14·3 | 121·3 ± 21·47 | 177·89 ± 19·42 |
| s (GP) | 119·45 ± 11·47 | 147·61 ± 27·22 | 150·92 ± 27·46 |
| cleus, Lateral (LHb) | 136·04 ± 10·17 | 155·23 ± 23·69 | 195·42 ± 22·79 |
| cleus, Medial (MHb) | 136·31 ± 8·83 | 166·48 ± 23·11 | 209·65 ± 22·81 |
| (Dentate) | 180·86 ± 9·98 | 224·11 ± 24·55 | 233·58 ± 18·67 |
| (pyramidal/molecular) | 160·18 ± 11·3 | 183·12 ± 19·7 | 228·78 ± 15·72 |
| , Anterior (AHy) | 102·09 ± 10·04 | 102.31 ± 15·18 | 187·8 ± 31·72 |
| , Lateral (LH) | 108·21 ± 5·08 | 123·75 ± 13·9 | 214·23 ± 21·37 |
| , Posterior (PH) | 109·08 ± 7·18 | 135·5 ± 12·79 | 214·2 ± 31·71 |
| ılus (Ic) | 135·63 ± 24·82 | 160·23 ± 14·65 | 194·85 ± 14·44 |
| cus (ll) | 77·53 ± 14·33 | 95·62 ± 5·38 | 188·43 ± 27·36 |
| ody, Posterior (MP) | 183·63 ± 25·6 | 218·62 ± 28·48 | 267·12 ± 32·65 |
| c Area (MPO) | 101·85 ± 6·69 | 108·31 ± 16·43 | 192·48 ± 35·46 |
| ex, Lateral (LOT) | 239·21 ± 25·9 | 378·45 ± 34·4 | 295·35 ± 33·46 |

| | | | |
|---|---|---|---|
| ve (ON) | 192·59 ± 15·12 | 278·72 ± 29·66 | 246·44 ± 30·81 |
| i (Pn) | 99·52 ± 22·66 | 146·49 ± 13·69 | 181·12 ± 28·35 |
| l Linear (RLi) | 112·94 ± 15·49 | 114·05 ± 22·48 | 188·12 ± 41·87 |
| R) | 125·91 ± 13·76 | 128·93 ± 30·67 | 163·09 ± 27·88 |
| s, Lateral (LS) | 108·6 ± 5·74 | 202·84 ± 16·85 | 202·78 ± 28·31 |
| gra, Reticular (SNR) | 102·95 ± 10·3 | 91·01 ± 14·59 | 151·24 ± 17·57 |
| gra, compacta (SNC) | 118·3 ± 10·76 | 104·34 ± 28·13 | 168·29 ± 23·77 |
| culus (SC) | 132·44 ± 14·39 | 124·29 ± 18·78 | 134·83 ± 25·40 |
| cleus, Dorsal (Dtg) | 140·74 ± 20·93 | 147·03 ± 20·83 | 172·93 ± 6·66 |
| leus, Lateral Posterior (LP) | 186·37 ± 16·36 | 207·04 ± 29·76 | 229·84 ± 19·75 |
| leus, Laterodorsal (LD) | 170·56 ± 15·65 | 231·54 ± 43·69 | 241·39 ± 30·95 |
| leus, Mediodorsal (MD) | 148·34 ± 10·95 | 196·35 ± 33·83 | 239·24 ± 17·92 |
| leus, Ventrolateral (VL) | 136·91 ± 25·56 | 219·44 ± 48·29 | 208·95 ± 23·12 |
| leus, Ventromedial (VM) | 150·41 ± 15·58 | 183·85 ± 39·71 | 192·39 ± 17·34 |
| sterior (Po) | 151·52 ± 13·47 | 205·71 ± 39·86 | 217·35 ± 19·03 |
| uniens (Re) | 131·67 ± 11·87 | 214·81 ± 45·06 | 252·19 ± 35·2 |

as are those identified in Paxinos, G. and Watson, C. *The rat brain in stereotaxic coordinates*, 2nd ed. Academic Press, S
represents the results of one-way ANOVAs and Newman-Keuls post hoc tests ($\alpha = 0{\cdot}05$) (See text). S = Sham; R = Micro

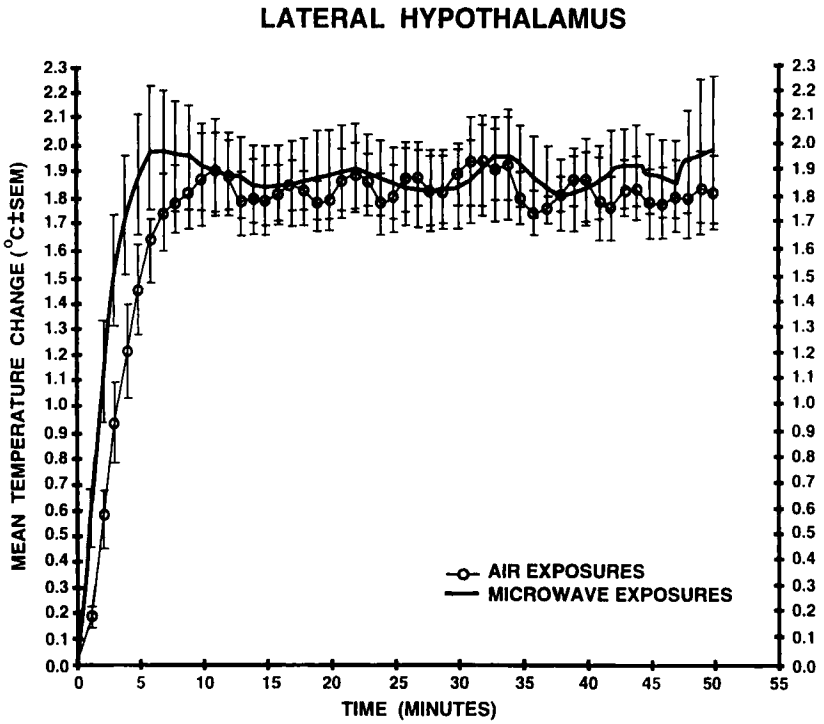


Figure 2. Temperature changes in the lateral hypothalamus (LH) while rats were exposed to hot/moist air or microwaves in a protocol similar to that used in Pilot Study 1. These data represent the findings of Pilot Study Number 2. These intracranial recordings revealed that, in the LH, where hot/moist air produced higher levels of LCGU than did microwaves (Table 1), the asymptote temperatures were not significantly different during exposure to the two hyperthermic agents. However, in these same animals, the rates of temperature rise were not well matched. Variance indicators are the standard error of the mean (SEM).

steady-state local brain temperatures and tympanic temperatures, the rates of temperature rise in the LH and DMC were slower in the rats exposed to hot air than they were in rats exposed to microwaves.

The above finding prompted us to perform a final parametric study (Pilot Study Number 3) in which we discovered how to adjust hot-air exposures to match both the rate of temperature rise and asymptote temperatures of the lateral hypothalamus of microwave-exposed ($N = 4$) or hot-air exposed ($N = 4$) rats. This was done by once again observing the relationship between tympanic membrane temperatures and temperatures in the LH during microwave or hot-air exposures. Once these relationships were established, we used the tympanic temperature as a reference point as we controlled microwave and hot air levels in anaesthetized rats injected with 2DG (see below).

Our new control techniques allowed good matching of both rates of temperature increase (mean rates = $0{\cdot}2 \pm 0{\cdot}05$°C/min for rats exposed to hot air; $0{\cdot}2 \pm 0{\cdot}03$°C/min for rats exposed to microwaves) and steady-state temperatures (mean plateau temperatures = $39{\cdot}9 \pm 0{\cdot}1$°C for rats exposed to hot air; $40{\cdot}0 \pm 0{\cdot}1$°C for rats exposed to microwaves) in the lateral hypothalamus (Figure 4). A repeated measure ANOVA revealed that there was not a significant difference ($\alpha = 0{\cdot}05$) between the

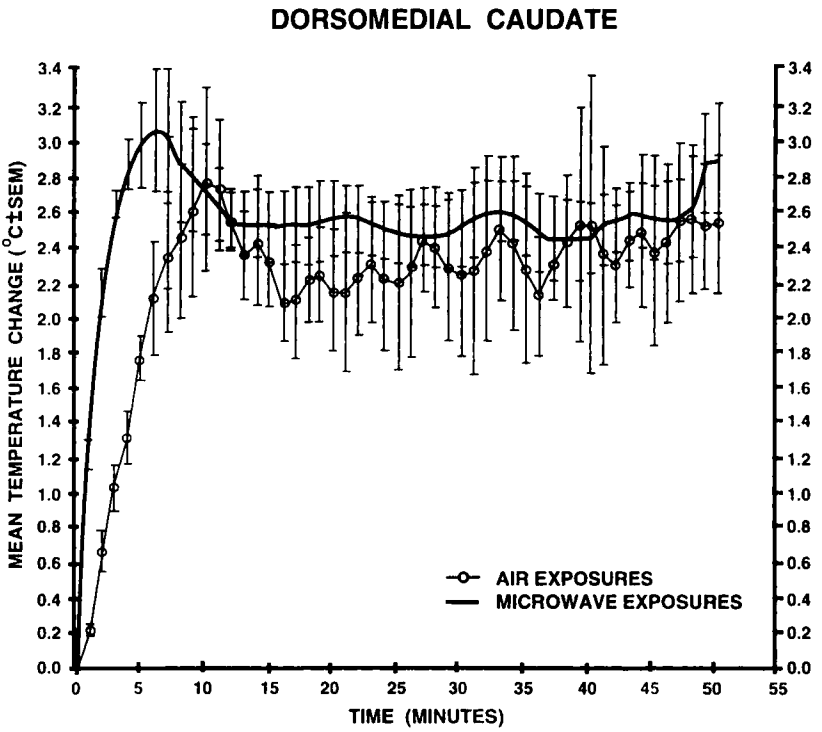


Figure 3. Temperature changes in the dorsomedial caudate (DMC) while rats were exposed to hot/moist air or microwaves in a protocol similar to that used for Pilot Study 1. These data represent the findings of Pilot Study Number 2. These intracranial recordings revealed that, in the DMC, where hot/moist air produced levels of LCGU comparable to those measured during microwave exposure (Table 1), the asymptote temperatures were not significantly different during exposure to the two hyperthermic agents. However, in these same animals, the rates of temperature rise were not well matched. Variance indicators asre the standard error of the mean (SEM).

LH temperatures (both during initial heating and during the steady hyperthermic state) of the microwave and hot-air exposed groups.

2.4. *Measurement of LCGU in hyperthermic rats*

The first set of LCGU measurements took advantage of the parameters developed in Pilot Study Number 1 where midbrain temperatures were matched during microwave or hot air exposure. The heads of anaesthetized (see regimen above) rats were exposed to either microwave irradiation ($N = 7$) or hot/moist air ($N = 7$; see above) in such a way that estimated midbrain temperature rose 2·0°C in 8·5 ± 0·9 min. Comparable tympanic temperature increases were 1·5 ± 0·3°C. Other rats were sham irradiated (anaethetized, placed in the exposure compartment, but not irradiated, $N = 7$).

During a second set of measurements, we used the data from our localized brain temperature determinations (Pilot Studies 2 and 3) to precisely control the rate and level of LH hyperthermia (2·0°C increase at a rate of 0·2°C/min). The pre-determined tympanic temperatures were reproduced and hypothalamic temperatures of microwave ($N = 4$) and hot/moist air exposed rats ($N = 4$) were matched and maintained for 45 min via our computerized temperature control system.

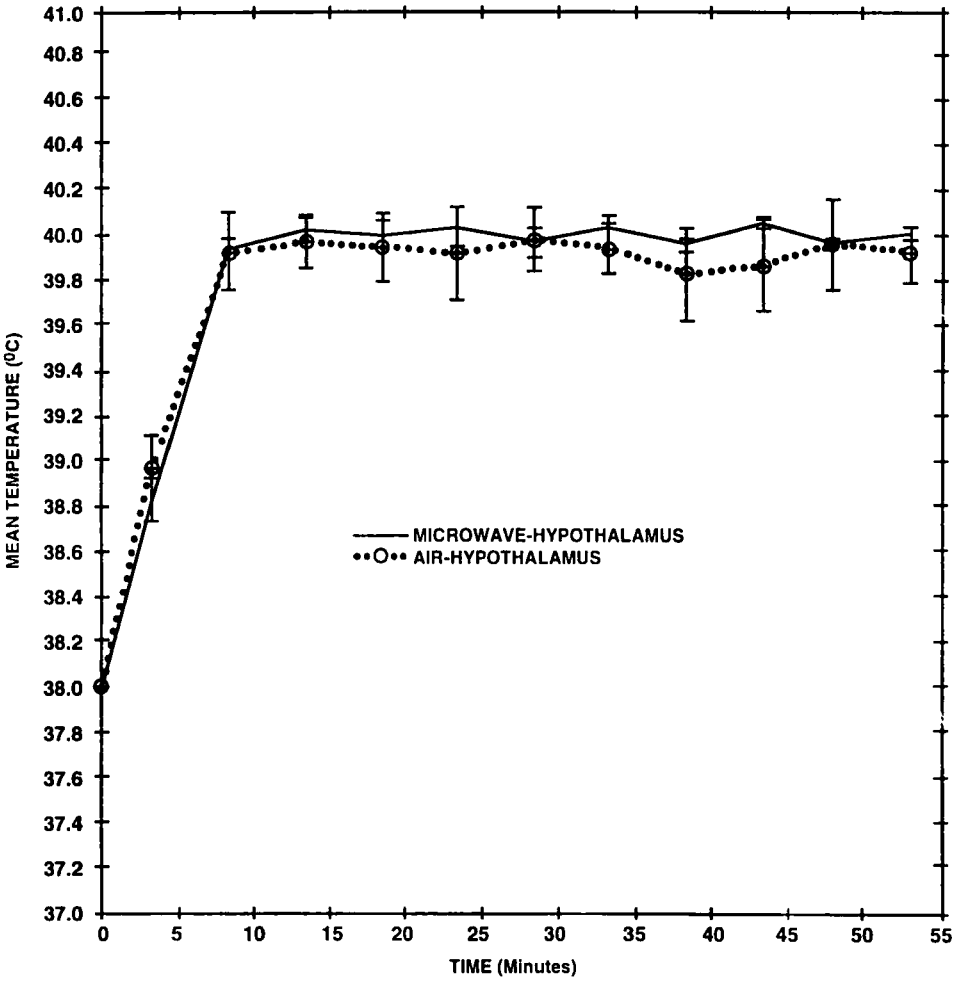


Figure 4. Temperature changes in the lateral hypothalamus during exposure to hot/moist air or microwaves. These data represent the findings of Pilot Study Number 3. The figure illustrates the results of procedural adjustments that allowed precise matching of both asymptote and rate of temperature increase during exposure to the two hyperthermic agents. Hypothalamic LCGU was not different between microwave- and air-exposed rats under these heating circumstances. Variance indicators are the standard error of the mean (SEM).

Methods for the determination of LCGU have been previously established by Sokoloff *et al.* (1977) and have been widely validated. Briefly, our rats were equipped with catheters in the femoral artery and vein under ketamine HCl (50 mg/kg, i.p.) and Acepromazine (10 mg/kg, i.p.) anaesthesia. The preanaesthetic agent Atropine Sulfate (0·4 mg/kg, i.p.) was also used to control salivation. We used non-metallic microwave-compatible temperature probes to record tympanic temperature, subcutaneous temperature (neck) and rectal temperature throughout the experiment. When the target tympanic temperature rise was achieved, rats received an i.v. injection of either 5·33 mCi/Kg Deoxy-D-glucose, 2-[1,2-$^{3}$H(N)]-$HOCH_2(CHOH)_3CH_2CHO$

(30–60 Ci/mmol) or 125 $\mu$Ci/Kg Deoxy-D-glucose, 2-[1-$^{14}$C]-$HOCH_2(CHOH)_3CH_2$-CHO (45–60 mCi/mmol) (obtained from DuPont Company, New England Nuclear, Boston, MA). Arterial blood sampling was initiated at the end of the injection and approximately 24, timed, 50–100 $\mu$l samples of arterial blood were collected during the 45 min exposure/sham-exposure period. The arterial blood samples were centrifuged to separate the plasma, which was frozen until assayed (for $^3$H or $^{14}$C 2DG concentrations) by liquid scintillation counting. Plasma glucose concentrations were measured by standard enzymatic methods (Sokoloff 1984). Immediately following this procedure rats were decapitated. The brains were rapidly removed and frozen in Freon XII maintained at −60°C with liquid nitrogen. When fully frozen, the brain was removed from the Freon XII and stored at −70°C until sectioned (20 $\mu$m). These brain sections were used to create autoradiographs by exposing the tissue to either Kodak SB film or Leica Ultrofilm. LCGUs were calculated using the plasma and local tissue concentrations of $^{14}$C or $^3$H according to the appropriate operational equation provided by Sokoloff *et al.* (1977). Optical scanning of radiographs and LCGU calculations were performed using an imaging system developed by the Drexel University Imaging Center (McEachron *et al.* 1987a,b).

## 3. Results

The data from the experiment where midbrain temperatures were matched during microwave or hot-air exposures (heating parameters similar to Pilot Study 1) indicated that both hyperthermic methods induced a general increase in brain metabolism. An overall analysis (Kirk 1982) comparing all 47 brain areas revealed that, in general, both microwave-exposed [$t(46) = -6{\cdot}78$; $p < 0{\cdot}001$] and hot-air exposed [$t(46) = -17{\cdot}71$; $p < 0{\cdot}001$] rats had significantly higher levels of LCGU than did sham-irradiated controls. Further, the brains of hot-air exposed rats exhibited, in general, higher LCGUs than did microwave exposed rats [$t(46) = -7{\cdot}117$; $p < 0{\cdot}0001$].

We calculated one-way analyses of variance (ANOVAs) comparing the LCGUs of selected brain areas of hot-air, microwave and sham-irradiated rats. When an ANOVA revealed a statistically significant difference ($\alpha = 0{\cdot}05$), Newman-Keuls post hoc tests ($\alpha = 0{\cdot}05$) allowed simultaneous comparisons of animals in the 3 treatment groups (Kirk 1982). Sixteen of the 47 brain areas sampled showed a statistically significant difference between the LCGUs of hyperthermic rats versus sham irradiated rats and/or hot-air exposed rats versus microwave exposed rats (Table 1). Microwave exposure did not produce significantly higher levels of local glucose utilization (compared to hot-air exposed hyperthermic controls) in any of the 47 brain areas sampled. However, hot-air exposed rats showed significantly higher LCGUs than microwave exposed rats in portions of the motor cortex, hypothalamus (anterior, posterior and lateral), lateral lemniscus and the substantia nigra (reticulata).

In a second set of animals we carefully matched the rate of LH temperature rise and peak temperatures achieved by our two hyperthermic methods and again measured hypothalamic LCGUs. We found that this precise matching eliminated the difference in hypothalamic LCGU (Table 1) following microwave or hot air exposure. Specifically, when gross midbrain temperature matching was achieved, hot-air exposure produced higher hypothalamic metabolic rates than did microwave radiation. However, LH LCGUs were essentially alike when microwave and hot-air exposures produced hypothalamic temperatures having similar rates of rise and plateaus (Table 2).

Table 2. Summary of Lateral Hypothalamic (LH) LCGUs ($\mu$M/100g/min) during either microwave or hot-air exposures. Also shown are the various rates of temperature rise (Mean ± SEM°C/min) and plateau temperatures employed (Mean ± SEM°C).

| Rate of temperature rise | Plateau temperature increase from baseline | Hot air LCGU | Microwave LCGU |
|---|---|---|---|
| 0·23 ± 0·05† | 1·92 ± 0·05 | 148·93 ± 12·81 | |
| 0·23 ± 0·03† | 2·0 ± 0·07 | | 153·79 ± 12·94 |
| 0·28 ± 0·03‡ | 1·81 ± 0·13 | 214·23 ± 21·37 | |
| 0·37 ± 0·05‡ | 1·87 ± 0·16 | | 123·75 ± 13·9 |

‡Data from the experiment when LH temperature rises were disparate.
†Data from experiment when LH temperature rises were similar.

## 4. Discussion

In order to evaluate the effects of microwave radiations on brain metabolism we developed a hot air control procedure to mimic the hyperthermia caused by 5·6 GHz microwave exposure. Initial use of this procedure revealed that microwaves and hot/moist air may be used to produce similar steady-rate hyperthermia treatments—as reflected by tympanic temperatures, midbrain temperatures and temperatures at specific nuclei. Using this paradigm we discovered that hyperthermia (produced by either agent) induced a general increase in brain metabolism. However, hot-air exposed rats showed significantly higher LCGU than microwaved exposed rats in portion of the motor cortex, hypothalamus (anterior, posterior and lateral), lateral lemniscus and the substantia nigra (reticulata). To the best of our knowledge, this is the first such direct comparison of nuclei-specific LCGU following different hyperthermic treatments. However, these data are consistent with other studies of LCGU in which rats were exposed to a single hyperthermic agent (McCulloch *et al.* 1982, Murakami and Morimoto 1982) in that particular nuclei (e.g. portions of the hypothalamus) seemed to be especially sensitive to temperature increases.

Additional studies showed that our initial procedures did not match rates of temperature increase at particular nuclei during the use of our two hyperthermic agents. Therefore, additional procedures were developed that allowed us to closely match both rate of temperature rise and steady-state temperatures in selected brain areas. We chose the LH as an initial test of this method and found that hypothalamic LCGU was not significantly different when rates of temperature increase, as well as asymptote temperatures, were made similar during microwave or hot/moist air exposure. These data are consistent with the hypothesis that microwaves exert their effects on brain metabolism through primarily thermal mechanisms. Given these data, it seems reasonable that microwave health and safety standards (see ANSI C95.1-1990) limit thermal loads rather than attenuate some as-yet-ill-described non-thermal effects.

It should not be surprising that hypothalamic functioning is responsive to temperature change. Previous studies have shown that increases in ambient temperatures or fever increase hypothalamic LCGU (McCulloch *et al.* 1982, Morimoto *et al.* 1986, Imai-Matsumura *et al* 1990). We now add hot/moist air and microwave exposure to the list of hyperthermic agents that alter hypothalamic metabolism. These data are consistent with the well-known role of hypothalamic neurons in thermoregulation (for review see Boulant 1991).

Our data may reflect a particular role for the hypothalamus in detecting

temperature change. This interpretation is supported by the data indicating that matching rates of LH temperature rise during hot/moist air or microwave exposure can eliminate the LCGU differences initially observed when rates of temperature increase were disparate. By comparison, different rates of temperature rise during hot air or microwave exposure did not cause different levels of LCGU in the DMC (a brain area not believed to be involved in thermoregulation).

It should also be noted that in both experiments where LH LCGUs were measured, the injection of 2-DG occurred after the peak tympanic/brain temperature had been achieved. These data suggest that LH metabolism may somehow reflect *previous* changes of local brain temperature rather than current steady state temperatures. It should also be recognized, however, that 2-DG concentrations in brain rise most rapidly in the first few minutes following an intravenous injection (Sokoloff *et al.* 1977). Thus our method of measuring glucose metabolism was perhaps most sensitive in reflecting glucose utilization in the period immediately following the induction of hyperthermia.

Changes in hypothalamic neuronal firing have been recorded when brain temperature is warmed or cooled to some target level (for review, see Hensel, 1981). However, neural codes that reflect rate of temperature change have not been fully explored. This may be due to the difficulty in differentiating the physiological response to achieving a particular temperature versus the physiological response to slow or rapid temperature changes. Nevertheless, Mercer and Jessen (1978) have recorded action potentials from single hypothalamic neurons of sheep during temperature rises and during steady state hyperthermic temperatures. In some cases, they discovered, single neurons fire during the temperature rise and then reduce firing rate during steady state temperatures. Further, Kobayashi's (1988) review of neural codes used by temperature-sensitive neurons in the hypothalamus, revealed that these neurons exhibit transient firing responses to rapid temperature changes. These electrophysiological data are consistent with our finding that LH metabolism is altered by rate of temperature change.

Our initial LCGU study showed that, in the LH, hot air produced slower rates of temperature increase than did microwave exposure (Figure 2) and that LCGU was higher in the hot-air exposed LH than in the microwave exposed LH (Table 1). When these data are combined with the data from the second study (when rates of LH temperature rise were matched—see Table 2) one can see that, up to a point, LCGU goes up along with rate of temperature increase. This trend is reversed at a rate of approximately 0·4°C/min when LH LCGU drops sharply. This result was unexpected but may reflect a pathological metabolic reaction to very rapid hyperthermia. This interpretation is consistent with Streffer's (1985) observation that glucose utilization is often reduced when hyperthermic stress is very strong. Likewise, Ho and Edwards (1977a,b) observed a decrease in oxygen consumption of mice that was most prominent after very large doses of microwaves (e.g. 44·3 W/kg).

These experiments illustrate some of the technical challenges that must be met in order to perform meaningful comparisons of the physiological effects produced by different hyperthermic agents. During the course of heating, rapidly changing thermal gradients are formed. These gradients reflect transient periods and locations of non-uniform temperatures with both different rates of temperature rise and different asymptotes. Since these gradients are transient, it is difficult to fully characterize the hyperthermic history of particular tissues *in vivo*. Our studies reinforce the importance of controlling for rate of temperature change as well as

steady state temperatures in thermal studies. Apparently, both of these factors can influence LCGU. When these factors are controlled, two points become clear: (1) significantly intense cephalic hyperthermia can enhance LCGU; and (2) microwave and hot-air-induced hyperthermia produce similar enhancements in LCGU. These brain metabolism data are consistent with an interpretation of microwave effects that characterizes microwave radiation as producing its neurophysiological effects primarily through heating (Klauenberg 1995).

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