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Brain Research, 187 (1980) 291–306

ALTERATIONS IN ACTIVITY AT AUDITORY NUCLEI OF THE RAT INDUCED BY EXPOSURE TO MICROWAVE RADIATION: AUTORADIOGRAPHIC EVIDENCE USING [^{14}C]2-DEOXY-D-GLUCOSE

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(Accepted August 30th, 1979)

Key words: microwave radiation — auditory system — autoradiography — [^{14}C]2-deoxy-D-glucose

SUMMARY

Autoradiographic maps of brain activity in rats exposed to pulsed or continuous-wave (CW) microwave radiation were made using [^{14}C]2-deoxy-D-glucose ([^{14}C]2-DG). Special emphasis was given to measurements of activity in the auditory system because previous work had shown that pulsed microwave radiation can elicit auditory responses in man and other animals. In particular, one middle ear was ablated in nine rats to attenuate the transmission of air-borne sound to one cochlea. The resulting imbalance in auditory input for four animals not exposed to microwave radiation was reflected as a bilateral asymmetry of [^{14}C]2-DG uptake at the inferior colliculus and medial geniculate body. In contrast, a symmetrical pattern of uptake at these structures in an animal exposed to *pulsed* microwave radiation showed that this stimulus bypasses the middle ear in eliciting auditory responses. This result established the utility of the [^{14}C]2-DG method for demonstrating a known effect of microwave radiation on brain activity. The results also revealed responses at auditory nuclei in 4 animals exposed to *CW* microwave radiation. These responses, which have not been observed with other methods, were evident at the power densities of 2.5 and 10 mW/sq. cm. To exclude the possibility that CW microwave radiation produced this result by direct action on brain tissue, additional data were obtained from two rats with one cochlea destroyed. In both animals, the uptake of [^{14}C]2-DG at the inferior colliculus and medial geniculate body was virtually identical to the uptake in animals not exposed to microwave radiation, i.e. greatest on the side of the brain contralateral to the intact cochlea. This finding, coupled with the finding of a bilateral symmetry of [^{14}C]2-DG uptake in the auditory pathways of animals with one middle ear ablated, confirmed the hypothesis that auditory responses to CW microwave radiation

originate within the cochlea. Effects on brain activity outside of the auditory system were not found in qualitative analyses of autoradiographs for the conditions of exposure to CW microwave radiation noted above or for exposure to pulsed microwave radiation at the average power density of 2.5 mW/sq. cm.

INTRODUCTION

Recent findings indicate that brain activity is altered during exposures of animals to non-ionizing radiation at average power densities of 10 mW/sq. cm or less. Among these findings are perception of pulsed microwave radiation as auditory sensations[17,22,25] and effects of microwave, very high frequency (VHF), and extremely low frequency (ELF) radiation on the electroencephalogram[1,6,23,52] and behavior[1,14,19,20,23,24,29,34,39,42,60]. Widely acknowledged mechanisms for the conversion of electromagnetic energy into heat or mechanical disturbances in tissue have lead to the suggestion that brain activity is altered via adequate stimulation of sensory receptors[16,40,50]. Indeed, numerous studies have provided direct or indirect evidence of effects of low-power, non-ionizing radiation on activity in the auditory[17,20,22,25,29,59,65], vestibular[19,37], and cutaneous[38,40] systems.

Many investigators now believe, however, that alterations in brain activity could result from radiation-induced changes in the immediate environment of neurons. In addition to possible effects of small increases in temperature on brain activity[41,61,63], such alterations might be evoked by changes in the electric fields around neurons[1,4,18,30,46,63] or in the biochemical composition of the intercellular space[1,2,21,44]. For example, exposures to ELF or amplitude-modulated VHF radiation can modify the binding of calcium ions to brain tissue but only over narrow ranges of amplitude and frequency for ELF exposure[5] or amplitude and modulation frequency for VHF exposure[7,8]. The absence of effects outside of these amplitude and frequency 'windows' argues strongly for a non-thermal mechanism of stimulation. Inasmuch as the binding and release of calcium have been linked to inhibition and excitation in the cerebral cortex[32], brain activity may be altered in animals exposed to radiation known to modify calcium binding in vitro.

The purpose of this paper is to describe the use of the [^{14}C]2-DG method for identifying the sites at which nonionizing radiation acts in eliciting brain responses. This method has an enormous sampling advantage over classical electrophysiological methods in that it allows for simultaneous determination of glucose utilization and associated functional activity in most macroscopic structures of the brain[33,47,51,53,56,57]. Thus, we expected that patterns of [^{14}C]2-DG uptake in the brains of exposed animals might reveal alterations in activity at sensory nuclei, resulting from stimulation of peripheral receptors, as well as other effects on brain activity, resulting from radiation-induced changes in neural environment.

To evaluate this application of the [^{14}C]2-DG method, brain activity was mapped in rats exposed to pulsed or CW microwave radiation. Measurements of activity in the auditory system were emphasized because results of psychophysical[17, [22,25], electrophysiological[10,59,65], and behavioral[29] studies have shown that pulsed microwave radiation can elicit auditory responses in man and other animals at average power densities far below 10 mW/sq. cm. Our results not only demonstrated this known effect of pulsed microwave radiation on brain activity but revealed heretofore unobserved responses at auditory nuclei in animals exposed to CW microwave radiation.

METHODS

Eleven Sprague–Dawley rats weighing between 150 and 250 g were used. Either one middle ear or one cochlea was destroyed in all animals to abolish or attenuate greatly the transmission of inputs to one side of the auditory system.

Surgery

Two or more days before stimulus exposure each of the first 9 animals were anesthetized with methoxyflurane, and a polyethylene catheter was inserted into the jugular vein. The free end of the cannula was routed subdermally to the back of the animal's neck where a small incision was made and then sutured. This incision later provided access to the catheter in order to inject [^{14}C]2-DG. Finally, the left bulla was opened, the ossicles were removed, and the bulla was packed with gelfoam.

One cochlea was destroyed in each of the remaining two animals by inserting a blunt probe through the round window. The [^{14}C]2-DG was administered via direct injection into a large tail vein, a procedure that produced results comparable to those obtained with the jugular catheter[51].

Exposure conditions

Exposures to all stimuli were carried out inside a double-walled sound isolation chamber (Industrial Acoustics Co. 1203A). During stimulus exposure, the animals were restrained in a cylindrical cage constructed of low-loss dielectric webbing. The cage was essentially transparent to microwave radiation, and its 1-cm mesh size was adequate to ensure unimpaired heat transfer between animal and environment. The sound isolation chamber was darkened for the period of stimulus exposure.

Immediately prior to stimulus exposure, each rat was injected with 25 μCi/100 g of body weight of [^{14}C]2-DG (New England Nuclear NEC-495) in a 1.5 ml volume of normal saline. The animals were then separately exposed to one of several stimuli for 45 min. Among the 4 animals not exposed to microwave radiation, two were exposed to acoustic clicks at 87 dB SPL per click; one was exposed to infrared radiation set at a level to mimic the total thermal load induced by microwave exposure at 10 mW/sq. cm; and one merely remained in the sound isolation chamber.

Acoustic clicks were produced by driving a loudspeaker (Acoustic Research AR-2) with 100 μsec, electronic pulses presented at the rate of 10/sec. The loudspeaker was located 1 m from the rats which were placed in a parallel-plate stripline with the same orientation used for exposures to CW microwave radiation.

Infrared radiation was generated by two heat lamps attached to opposite sides of the stripline. The voltage supplied to the lamps was adjusted so that the rate of temperature increase, measured at a point within a saline-filled beaker during exposure to infrared radiation, matched the increase measured in the same beaker during exposure to 918 MHz radiation at 10 mW/sq. cm. The cross-sectional area of the beaker approximated that of test rats. Thus, a control was provided for the total thermal load experienced by animals exposed to microwave radiation at 10 mW/sq. cm. We must note, however, that the spatial profile of microwave-induced heating was not reproduced by exposure to infrared radiation.

Seven animals were exposed to microwave radiation. In an initial experiment, one rat was exposed to pulsed microwave radiation at the average power density of 2.5 mW/sq. cm and the peak power density of 12.5 W/sq. cm. Pronounced differences in the patterns of [^{14}C]2-DG uptake at auditory nuclei between this animal and controls established the utility of the [^{14}C]2-DG method for demonstrating a known effect of microwave radiation on brain activity. With this assurance, two rats each were used to identify possible alterations in activity induced by exposures to CW microwave radiation at 2.5 and 10 mW/sq. cm. Results obtained from these animals revealed unexpected responses at the inferior colliculus and medial geniculate body. To localize the site of CW microwave action in stimulating the auditory system, two additional rats with one cochlea destroyed were exposed to CW microwave radiation at 10 mW/sq. cm.

For the case of exposure to pulsed microwave radiation, 20 μsec pulses of 2450 MHz energy were fed to a rectangular-waveguide horn antenna (DeMornay-Bonardi DBI-520) at the rate of 10/sec. The antenna was located 8 cm from the rat's head, placing the animal in the fringe zone of the radiation field where conditions of plane-wave exposure first occur. Densities of average power were measured with an isotropic field-intensity probe (General Microwave Corp., Raham model 2) at the position in the field normally occupied by the rat. These measurements, along with the duty factor of the modulation waveform, were used to calculate densities of peak power. The microwave source (Applied Microwave Laboratory PH40K) was located outside of the sound isolation chamber.

Animals were exposed to CW microwave radiation in the parallel-plate stripline, which was excited by a 918 MHz microwave source (Airborne Instrument Laboratory 125) and terminated by a nonreflecting load. The output of the microwave source was monitored through a directional coupler and associated power meter (Hewlett-Packard 435A). Individual rats were placed on the center conductor of the stripline so that their heads were facing the impinging radiation and so that the long axis of their bodies was aligned with the direction of wave propagation. Densities of incident power at the rat were calculated from the known characteristics of the stripline[31] and the output of the microwave source. The accuracy of these calculations was confirmed by direct measurements of field intensity with the Raham probe. The microwave source, directional coupler, and power meter were located outside of the sound isolation chamber.

Densities of absorbed power in animals exposed to CW microwave radiation were estimated using a thermometric technique. Briefly, the carcass of a 170 g rat was placed on the center conductor of the stripline in a manner identical to that of test animals. A temperature sensor (Ramal, Inc. model LCT-1), especially designed to minimize the perturbation of specific absorption rates (SARs) in tissue[48], was then inserted into the midbrain through an opening made in the skull. The initial rate of temperature increase induced by exposure to 918 MHz radiation at 100 mW/sq. cm was recorded and subsequently related to SAR with the equation[28]:

$$SAR = K\varrho c \Delta T/t,$$

where SAR is the density of absorbed power in W/cc, K is 4.186 joules/cal, ϱ is the tissue density in g/cc, c is the specific heat of the tissue in cal/g/°C, and $\Delta T/t$ is the initial rate of temperature increase in °C/sec. After substituting the values of 1.05 g/cc and 0.88 cal/g/°C for the density and specific heat of brain tissue[13], we found that the SAR in the midbrain of the rat carcass was 0.46 mW/cc per mW/sq. cm of incident power. SARs in the midbrains of test rats were probably slightly different due to inaccuracies in the measurements and small differences in animal size.

Measurement of [^{14}C]2-DG uptake in the brain

Animals were killed at the end of the stimulus exposure period with an overdose of Nembutal. The brains were removed as rapidly as possible, frozen in isopentane cooled to —79 °C, and placed in a cryostat set at —20 °C. After the temperature of the brain rose to that of the cryostat, the brain was sectioned at 30 μm in the frontal plane. The sections were lifted from the microtome knife with prewarmed coverslips and immediately dried at 60 °C on a hotplate. Autoradiographs of [^{14}C]2-DG uptake throughout the brain were produced by apposing the sections to the emulsion side of X-ray film, which was developed after 5 days of exposure for coarse-grain film (Kodak SB-54) or after 12–14 days for fine-grain film (Cronex Lo'dose mammography film).

To identify stimulus-induced alterations in brain activity, the autoradiographs were examined for differences in optical densities between the different conditions of stimulus exposure. In addition, each X-ray film was visually scanned to locate representative sections through the auditory and vestibular nuclei in all brains. Autoradiographs selected according to these criteria were photographically enlarged, and the sections from which they were made were stained with cresyl violet. In this way, regional measures of [^{14}C]2-DG uptake could be related to the cytoarchitecture shown in the stained sections.

RESULTS

The principal findings of this study were revealed by the procedure of ablating either one middle ear or one cochlea. Autoradiographs obtained from animals prepared in this way exhibited pronounced differences in the patterns of optical density at auditory nuclei for different conditions of stimulus exposure. Because these differences were most evident at the inferior colliculus (Fig. 1), results at this level of the brain will be described in detail. The responses at the inferior colliculus in animals exposed to control stimuli will be contrasted with the responses in animals exposed to microwave radiation. Finally, results at other brain structures will be mentioned.

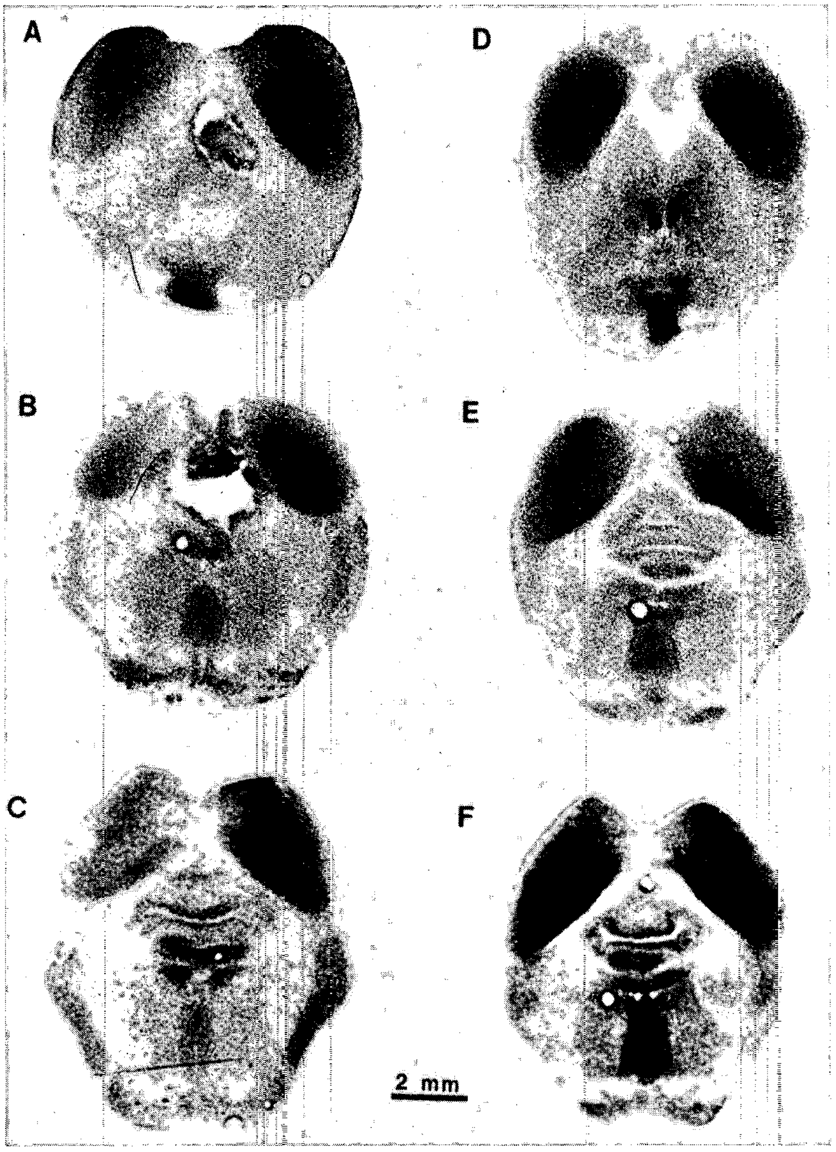

Fig. 1. Autoradiographs of [^{14}C]2-DG uptake at the level of the inferior colliculus. Autoradiographs obtained from animals exposed to control stimuli are presented in the left column and include representative sections for cases of exposure to acoustic clicks (A), no radiation (B), and infrared radiation (C). Autoradiographs obtained from animals exposed to microwave radiation are presented in the right column and include representative sections for cases of exposure to pulsed microwave radiation at the peak and average power densities of 12.5 W/sq. cm and 2.5 mW/sq. cm (D), CW microwave radiation at 10 mW/sq cm (E), and CW microwave radiation at 2.5 mW/sq. cm (F). Prior to stimulus exposure, the middle ear ossicles were ablated in the ear on the right-hand side of the sections. Cortex is not included in the autoradiographs presented in this figure. The cytoarchitecture of sections at the level of the inferior colliculus is indicated in the line drawing presented in Fig. 2C. The small circles in some of the autoradiographs are artifacts produced by the formation of air bubbles during the transfer of sections from cryostat to coverslips.

Patterns of [¹⁴C]2-DG uptake at the inferior colliculus in animals exposed to control stimuli

An autoradiograph from an animal exposed to acoustic clicks is shown in Fig. 1A. There is an obvious asymmetry of optical densities in this autoradiograph, with the darkest area at the central nucleus of the inferior colliculus on the side of the brain contralateral to the intact middle ear. This result was expected in that most ascending pathways from one cochlea ultimately lead to the central nucleus of the contralateral inferior colliculus[11,45,64] and in that similar patterns of [^{14}C]2-DG uptake at the inferior colliculus had been reported by other investigators who used animals with one ear canal occluded[33,47,56] or one cochlea destroyed[58].

A bilateral asymmetry of [^{14}C]2-DG uptake at the inferior colliculus was also observed in the two control animals not deliberately exposed to sound. This result is demonstrated in Fig. 1B, C, where Fig. 1B shows an autoradiograph from the animal that merely remained in the sound isolation chamber for the period of stimulus exposure, and Fig. 1C shows an autoradiograph from the animal exposed to infrared radiation. The pattern of optical densities present in both autoradiographs indicates that weak stimulation of one cochlea, via conduction of ambient sound through the intact middle ear, was sufficient to evoke a strong metabolic response in the contralateral inferior colliculus. There appears, in fact, to be little difference in optical densities at the inferior colliculus contralateral to the intact middle ear between animals exposed to clicks (Fig. 1A) and animals not exposed to clicks (Fig. 1B, C). This comparison suggests not only that relative increases in the uptake of [^{14}C]2-DG at one inferior colliculus are a sensitive indicator of input from the contralateral cochlea but that the dynamic range of graded responses to stimuli above the threshold of measurement may be somewhat limited.

Patterns of [^{14}C]2-DG uptake at the inferior colliculus in animals exposed to microwave radiation

In contrast to the picture at the inferior colliculus for controls, a symmetrical uptake of [^{14}C]2-DG was found in animals exposed to microwave radiation. An autoradiograph obtained from the animal exposed to *pulsed* microwave radiation is shown in Fig. 1D. In this autoradiograph, the optical density at one inferior colliculus is equal or nearly equal to that at the other inferior colliculus, and the optical densities at both inferior colliculi approximate those of the most dense inferior colliculus in control animals (Fig. 1A–C). This result indicates that pulsed microwave radiation can elicit a metabolic response in the central auditory system by some mechanism other than conduction of energy through the middle ear. Psychophysical and electrophysiological observations are consistent with this interpretation. For example, auditory responses to pulsed microwave radiation have been demonstrated in human subjects who had severe losses of middle-ear function[17] and in guinea pigs with interrupted ossicular chains[10].

Patterns of [^{14}C]2-DG uptake for cases of exposure to *CW* microwave radiation were surprisingly similar to those observed for the case of exposure to pulsed microwave radiation. Autoradiographs are presented in Fig. 1E for an animal exposed to

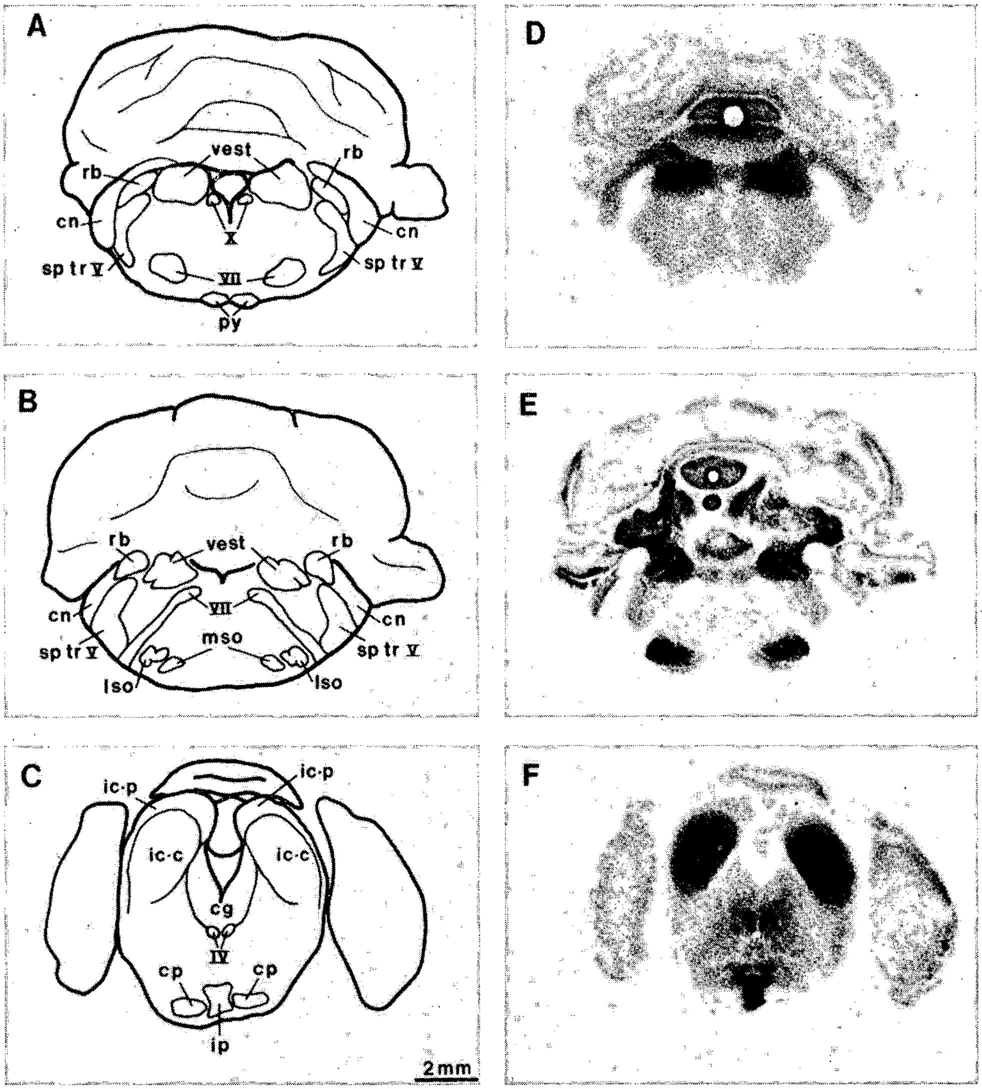

Fig. 2. Autoradiographs of [^{14}C]2-DG uptake at 3 levels in the brain of a rat exposed to pulsed microwave radiation. Autoradiographs are presented in the right column (D, E, and F) and are arranged in a caudal to rostral order. Line drawings used to identify structures in the sections are shown to the immediate left of each corresponding autoradiograph. Abbreviations: cg, central gray; cn, cochlear nucleus; cp, cerebral peduncle; ip, interpeduncular nuclei; ic.c, central nucleus of the inferior colliculus; ic.p, pericentral zone of the inferior colliculus; lso, lateral superior olive; mso, medial superior olive; IV, nucleus of the trochlear nerve; VII, facial nerve; py, pyramids; rb, restiform body; sp tr V, spinal tract of the trigeminal nerve; vest, vestibular nuclei; X, motor nucleus of the vagus nerve.

CW microwave radiation at 10 mW/sq. cm and Fig. 1F for an animal exposed at 2.5 mW/sq. cm. These autoradiographs show a bilateral symmetry of optical densities at the inferior colliculus, indicating an auditory response to CW microwave radiation.

To exclude the possibility that CW microwave radiation produced this result by direct action on brain tissue, additional data were obtained from two animals in which one cochlea was destroyed. In both animals, the uptake of [^{14}C]2-DG was greatest at

Note: The text on p. 299 was printed out of sequence; it should be skipped when reading the RESULTS section and should be inserted between pp. 300 and 301 when reading the DISCUSSION section.

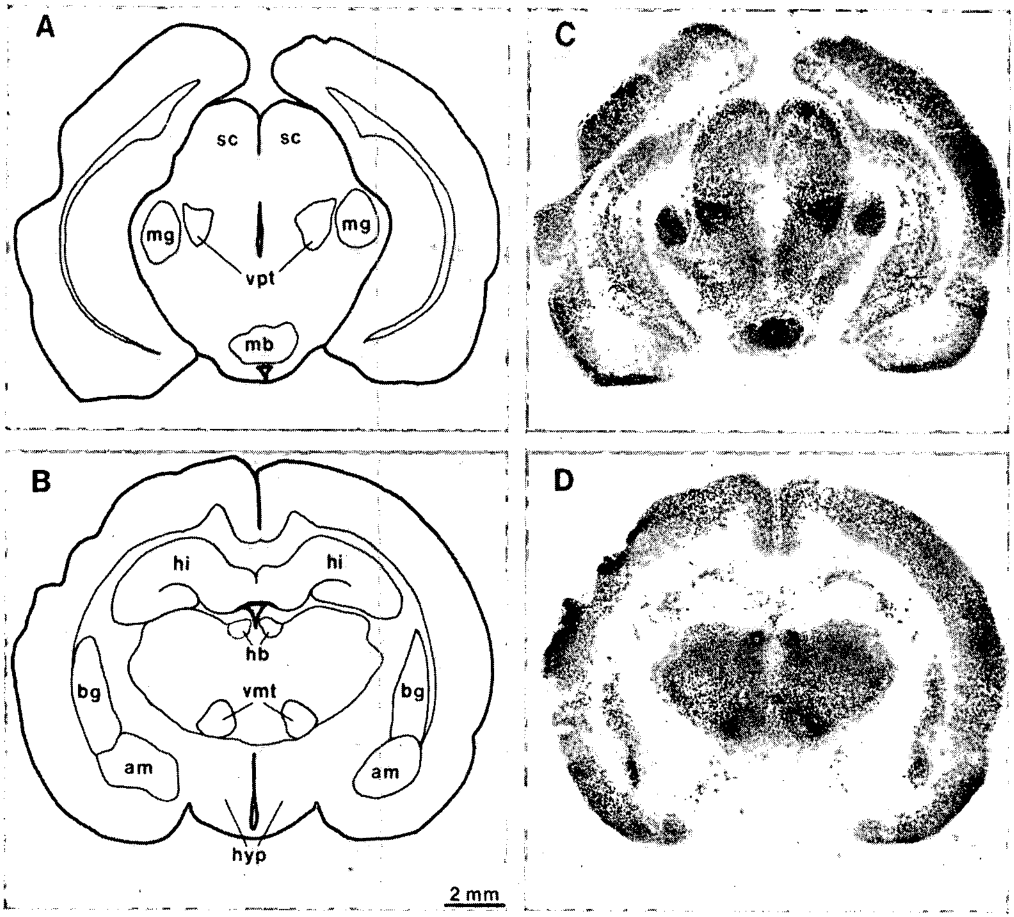

Fig. 3. Autoradiographs of [^{14}C]2-DG uptake in rostral sections obtained from the brain of a rat exposed to pulsed microwave radiation. Autoradiographs of caudal sections from this brain are presented in Fig. 2. Abbreviations: am, amygdala; bg, basal ganglia; hb, habenula; hi, hippocampus; hyp, hypothalamus; mb, mammillary bodies; mg, medial geniculate body; sc, superior colliculus; vmt, ventral medial thalamic nuclei; vpt, ventral posterior thalamic nuclei. The scallops and folds on the periphery of both autoradiographs are cutting artifacts.

glucose utilization and associated functional activity in most macroscopic structures of the brain, effects on the activity of nuclei in sensory systems, resulting from stimulation of peripheral receptors, can be identified and separated from other effects on brain activity, resulting from radiation-induced changes in neural environment. Moreover, the interpretation of autoradiographs of [^{14}C]2-DG uptake in the brains of animals exposed to electromagnetic radiation cannot be confounded by the artifacts that are often present in recordings of electrical activity obtained under the same conditions of exposure[28]. Finally, the [^{14}C]2-DG method appears to be especially useful for the detection of slowly-varying or continuous alterations in brain activity in that rates of glucose utilization are 'integrated' over a 45 min period of stimulus exposure. This last advantage may be crucial in demonstrating effects of CW microwave radiation on brain activity.

the inferior colliculus contralateral to the intact cochlea. The degree of asymmetry at the inferior colliculus was, in fact, at least as great as that found in any of the control animals (Fig. 1A–C). This finding, coupled with the finding of a bilateral symmetry of [^{14}C]2-DG uptake in the auditory pathways of animals with one middle ear ablated, demonstrated that CW microwave radiation acts at some site within the cochlea in eliciting auditory responses.

Patterns of [^{14}C]2-DG uptake at other brain structures

While the patterns of [^{14}C]2-DG uptake at the inferior colliculus revealed most clearly the differences in the responses of the auditory system to microwave and control stimuli, the autoradiographic appearance of other auditory nuclei will be mentioned. Auditory nuclei caudal to the inferior colliculus exhibited high optical densities in all brains. Representative autoradiographs at the levels of the cochlear nucleus and superior olivary complex are presented in Fig. 2D, E. Although the characteristic shapes of the lateral superior olive, medial superior olive and cochlear nucleus can be discerned by comparing autoradiographs with line drawings prepared from Nissl stains (Fig. 2A, B), bilateral differences were not observed except in autoradiographs obtained from the two animals with one cochlea destroyed.

Within the medial geniculate body, optical density was greatest in the ventral divisions for all conditions of stimulus exposure, and the relative uptake of [^{14}C]2-DG between the two sides of the brain paralleled that at the inferior colliculus. This result is not surprising since the major projections from one inferior colliculus are to the ventral division of the ipsilateral medial geniculate body. Fig. 3C shows the pattern of optical density at the level of the medial geniculate for the animal exposed to pulsed microwave radiation.

Outside of the auditory system, no qualitative differences in optical densities were found between exposure conditions. Many of the features of autoradiographs that were essentially the same from brain to brain can be seen in Figs. 2 and 3. These figures show results at 5 levels in the brain of the rat exposed to pulsed microwave radiation. The patterns of optical density in autoradiographs throughout this brain are similar to those reported by Schwartz and Sharp[51] for resting, awake rats. Structures exhibiting high optical densities in all brains included the vestibular nuclei (Fig. 2D), deep cerebellar nuclei (Fig. 2E), dorsal cochlear nucleus (Fig. 2D and E), lateral superior olive (Fig. 2E), medial superior olive (Fig. 2E), interpeduncular nuclei (Fig. 2F), nucleus of the IVth cranial nerve (Fig. 2F), mammillary bodies (Fig. 3C), a portion of the amygdala (Fig. 3D), and the ventral medial thalamus (Fig. 3D). Chief among the structures exhibiting low optical densities were the fiber tracts (Fig. 2D, E) and hypothalamus (Fig. 3D).

DISCUSSION

Efficacy of the [^{14}C]2-DG method

The results of this study indicate that the [^{14}C]2-DG method can be a powerful tool for identifying the sites at which nonionizing radiation acts in eliciting brain responses. Because this method allows for simultaneous, in vivo determination of

A limitation of the [^{14}C]2-DG method is an apparent restriction in the dynamic range of measurement. Unless special procedures are used to eliminate or greatly reduce all extraneous inputs, the high level of 'background' activity in sensory systems can easily mask stimulus-evoked responses. In the present study, for example, one middle ear was ablated to attenuate the transmission of air-borne sound to one cochlea. The high optical densities found in autoradiographs at central auditory nuclei contralateral to the intact ear were always at or near the upper end of the dynamic range of measurement, even for the control animals not exposed to acoustic clicks. Thus, the removal of one middle ear was essential in detecting auditory responses to microwave radiation.

Unfortunately, the limitation in dynamic range precluded the evaluation of effects that might result from microwave stimulation of vestibular receptors. The optical densities in autoradiographs at vestibular nuclei were uniformly high in all brains of this study. Presumably, the activity reflected in these autoradiographs was at least a partial result of uncontrolled stimulation of the vestibular end-organ during small movements of the animal's head. This conclusion is supported by the findings of Sharp[53] who reported relatively low levels of optical density in autoradiographs of [^{14}C]2-DG uptake at the vestibular centers in the medulla and cerebellum of immobile rats. Completely immobile preparations will be needed in future studies to evaluate the possibility of microwave-evoked responses in the vestibular system.

Possible mechanisms for the effects of microwave radiation on auditory activity

While several explanations have been offered for the effect of *pulsed* microwave radiation on auditory activity, the only explanation for which there is much experimental support is that stimulation of the cochlea occurs via microwave-induced waves of intracranial pressure. Such mechanical stresses could be produced in irradiated tissue by 'thermoelastic' expansion during rapid absorption of electromagnetic energy, by electrostriction, or by radiation pressure[16,25,40,50,54]. Recent recordings of single-fiber activity in the cat's auditory nerve suggest, in fact, that a majority of responses to pulsed microwave radiation are the result of mechanical stresses induced at or peripheral to the basilar membrane[65].

An unexpected finding of the present study is the sensitivity of the auditory system to *CW* microwave radiation. Mechanical stimulation of the cochlea seems to be an unlikely explanation for this finding because our animals were exposed to CW microwave radiation with an initial rise time of about 1 min. This gradual application of electromagnetic energy falls far short of the minimum rise time (approximately 1 μsec) needed to produce measureable stresses in solid materials[25,54] or in containers of KCl solution[16] during exposure to pulsed microwave radiation. Moreover, responses to CW microwave radiation were clearly evident at the incident power density of 2.5 mW/sq. cm; a level more than 30 times lower than the thresholds of peak power established for auditory responses to microwave pulses in man[22,25] and other animals[25]. These responses to CW microwave radiation, along with certain responses to pulsed microwave radiation not readily explained by the hypothesis of mechanical stimulation[62,65], indicate that at least two mechanisms are involved in the effects of microwave radiation on auditory activity.

Results obtained in this study demonstrate that CW microwave radiation acts at some site within the cochlea in eliciting auditory responses. To speculate briefly on possible mechanisms of stimulation, we note that the processes of signal detection and transmission in the cochlea are affected not only by the presence of mechanical stimuli but by alterations in temperature[12,55], passage of extrinsic currents across the organ of Corti[26,27,36] and changes in the concentration of calcium ions in the perilymph[15,35,43]. As mentioned before, conversion of electromagnetic into thermal energy is a certain consequence of exposure to microwave radiation. Based on long-term measurements of microwave-induced heating in the midbrain of a 170 g rat carcass (see Methods) and considering the low rate of volumetric blood flow in the cochlea[3], we estimate a steady-state increase in intracochlear temperature of between 0.1 and 0.5 °C occurs in live rats exposed to 918 MHz radiation at 2.5 mW/sq. cm. The amplitude and latency of gross responses to clicks in the auditory nerve of cats are, to a good approximation, linearly dependent on intracochlear temperature over the range of 0—45 °C[12]. In recordings from single fibers in the auditory nerve, a 4 °C increase (from 34 to 38 °C) doubles the rate of responses to tone bursts, produces a 0.04 octave increase in the best frequency of monitored neurons and enhances spontaneous activity in the absence of tonal stimuli[55]. These findings suggest that even small increases in temperature produced during exposure to low-power microwave radiation may be effective in altering auditory activity.

In addition to the increases in temperature noted above, the cochlea may also be sensitive to electrical stimuli produced at cell membranes in the presence of microwave fields. Both spontaneous and evoked activity in the auditory nerve are increased when extrinsic currents are passed through the organ of Corti in the direction of scala vestibuli to scala tympani[36]. Studies of cochlear models indicate that the threshold of responses to injected current across the cilia-bearing ends of individual hair cells may be well below 5×10^{-12} A[26]. Strong asymmetries in charge between the layers of cell membranes and extracellular fluid have led to the suggestion that microwave-frequency fields can be rectified — albeit inefficiently — at such boundaries in tissue[1,4,30,63]. Alternatively, resonant interactions between the electromagnetic field and gating particles within membrane channels could displace or 'flip' the particles to their open configurations, thereby allowing ions to penetrate the membrane[46]. In either case, effects of microwave-induced currents might first be observed within the cochlea where the hair cells are extremely sensitive to perturbations in ionic flux.

Finally, it is intriguing to note a possible correlation between radiation-induced changes in the binding of calcium ions to brain tissue and the known sensitivity of the cochlea to changes in the concentration of calcium ions in the perilymph. In particular, increased calcium in the perilymph mimics the inhibitory action of olivocochlear bundle (OCB) stimulation in that the cochlear microphonic is augmented while the whole-nerve action potential is depressed[43]. This and other findings[15,35] suggest that calcium plays an essential role in the release of transmitter substance at the terminations of OCB fibers. If exposure to microwave radiation changes the concentration of calcium ions at these, or perhaps other, sites within the cochlea, then alterations in auditory activity might be expected. At this writing, however, radiation-induced changes in calcium binding have been observed only over narrow ranges of amplitude and frequency for ELF exposure[5] or amplitude and modulation frequency for VHF exposure[7,8]. Possible changes induced by exposure to CW microwave radiation therefore appear to be remote.

Perception of CW microwave radiation

The effect of CW microwave radiation on auditory activity raises the question of whether or not this form of energy can be perceived. Although no reports of *direct* sensation have appeared in the literature, Russian investigators long ago maintained that shifts in auditory threshold occur during or shortly after exposure to low-power, CW microwave radiation (for a review of this early work, see refs. 9, 34 and 40). More recently, Sagalovich and Melkumova[49] have reported a decline in the magnitude of click-evoked potentials at the auditory cortex of rabbits and white mice when the animals were exposed to pulsed microwave radiation. Significant decreases were seen at the peak power densities of 14 and 28 mW/sq. cm; levels well below that (80 mW/sq. cm) needed to elicit 'direct' auditory sensations[22,25]. In another study, Bourgeois found increases in the auditory sensitivity of human subjects during exposure to CW or sine-wave modulated microwave radiation at low densities of peak and average power (the maximum power density used was 2.1 mW/sq. cm)[9]. While these results are difficult to interpret because Sagalovich and Melkumova used metal electrodes to monitor brain potentials in an electromagnetic field[28] and because the increases in sensitivity found by Bourgeois were quite small, they are consistent with the three hypotheses outlined above in that each of these hypotheses predict shifts in auditory threshold. Thus, the presence of CW microwave energy may be cued indirectly via perceived changes in the loudness of environmental sounds.

Directions for future research

The results of this study demonstrated radiation-induced alterations in the activity of the auditory system. No obvious changes were found elsewhere in the brain. In view of the evidence cited in the Introduction, however, a true absence of effects outside of the auditory system seems unlikely. Quantitative, rather than qualitative, analyses of autoradiographs may reveal such effects in future studies. A wider range of exposure conditions might also help to identify additional structures in the brain that are sensitive to low-power, nonionizing radiation. For example, the [^{14}C]2-DG method could be particularly useful in investigations to define the functional correlates, if any, of the modifications in calcium binding observed immediately after exposures to ELF or amplitude-modulated VHF radiation. Finally, careful elimination of extraneous inputs will be needed to evaluate the possibility of microwave-evoked responses in the vestibular system.

ACKNOWLEDGEMENT

This study was supported by the National Institute of Environmental Health Sciences.

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