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Effects of Low-Level Microwave Irradiation on Hippocampal and Frontal Cortical Choline Uptake are Classically Conditionable

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LAI, H., A. HORITA, C. K. CHOU AND A. W. GUY. *Effects of low-level microwave irradiation on hippocampal and frontal cortical choline uptake are classically conditionable.* PHARMACOL BIOCHEM BEHAV 27(4) 635–639, 1987.—In previous research, we found that sodium-dependent high-affinity choline uptake in the hippocampus and frontal cortex of the rat was lowered after acute (45 min) exposure to low-level 2450-MHz pulsed microwaves (power density 1 mW/cm^2; average whole body specific absorption rate, 0.6 W/kg; 2 μsec pulses, 500 pps). In the present experiment, we investigated developments of tolerance and classical conditioning to these effects of microwaves. Rats were exposed to microwaves in cylindrical waveguides in 10 daily sessions (45 min per session). In an 11th session, we subjected the rats to either microwave (study of tolerance) or sham exposure (study of conditioned effect) for 45 min, and immediately measured choline uptake in the hippocampus and frontal cortex. We found that tolerance, a decrease in response to microwaves, developed to the effect of microwaves on choline uptake in the hippocampus, but not in the frontal cortex. Conditioned effects were also observed: an increase in choline uptake in the hippocampus and a decrease in uptake in the frontal cortex. These data suggest that the effects of microwaves on choline uptake in the hippocampus and frontal cortex are classically conditionable, probably to cues in the exposure environment.

Microwaves Choline uptake Hippocampus Frontal cortex Classical conditioning

HIGH power continuous-wave as well as modulated microwaves are being widely used as a source of heating for industrial processing of materials and food, microwave ovens and medical therapy. Also low to high power continuous-wave, modulated, and pulsed microwaves are extensively used for air-traffic control systems, police and military radars, earth to satellite television broadcast systems, and long-distance telephone communications. The possible hazardous effects of accidental and work-related exposure to the radiation are a concern. Particularly, the biological effects of acute and repeated exposure to microwaves at low power densities and different modulation characteristics need investigation. In previous research, we found that low-level pulsed microwave irradiation affects the functions of the central nervous system. Rats, after acute exposure to pulsed microwaves, responded differently to a variety of psychoactive drugs including apomorphine, amphetamine, morphine, ethanol, and pentobarbital [8–10]. Endogenous opioids appear to mediate some of these effects since they were blocked by pretreatment with narcotic antagonists. Recently, we found that acute pulsed microwave exposure decreased sodium-dependent high-affinity choline uptake, an index of activity of cholinergic innervations [21], in the hippocampus and frontal cortex in the rat [14]. These effects were selective since no significant effect was observed in the hypothalamus and striatum after acute microwave exposure.

Furthermore, in a series of experiments, we found that the effects of pulsed microwaves are classically conditionable [11–13]. In these experiments, rats were exposed in waveguides to pulsed microwaves in 10 daily sessions. In an additional session, they were sham exposed, i.e., they were placed in the waveguide but not subjected to the irradiation. A conditioned response was observed after the sham-exposure session. Apparently, the effects of microwaves became classically conditioned to cues in the exposure environment, which became the conditioned stimuli.

In the present experiment, we investigated whether the effects of pulsed microwaves on choline uptake in the hippocampus and frontal cortex are also classically conditiona-

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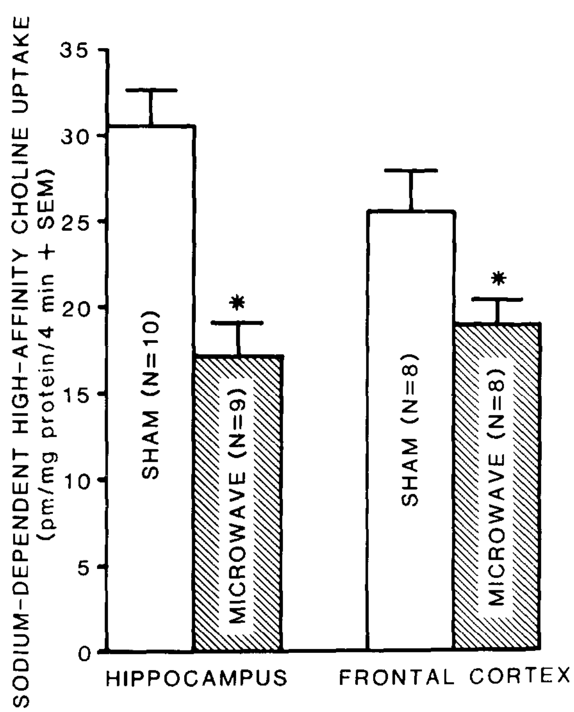

FIG. 1. Effects of acute (45 min) exposure to microwaves on choline uptake in the hippocampus and frontal cortex. Shaded bar= microwave exposed; open bar=sham exposed. Data were compared by the two-tailed Student's t-test. *Significantly different from response of sham-irradiated rats at $p<0.01$.

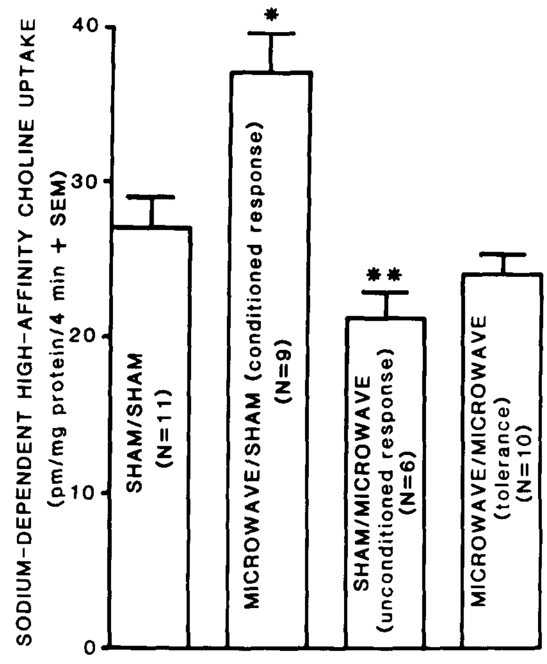

FIG. 2. Choline uptake in the hippocampus of rats in the different exposure-treatment groups assayed after exposure session 11. One-way ANOVA of the data showed a significant treatment effect: $F(3,32)=9.89$, $p<0.01$. *,**Significantly different from response of the sham/sham rats at $p<0.01$ and 0.05, respectively.

ble. We irradiated rats with pulsed microwaves at an average whole-body specific absorption rate of 0.6 W/kg of body weight. This level of power absorption is 1.5 times that of the human exposure safety standard recommended by the American National Standards Institute [19]. It causes no significant change in colonic temperature after 45 min of exposure. Apparently, the rats were able to dissipate the heat load and maintain constant body temperature at this level of irradiation.

METHOD

Animals

Male Sprague-Dawley rats (weighing 250–300 g at the start of the experiment) from Tyler Laboratory, Bellevue, WA were used. They were housed four to a cage in a temperature-controlled (22°C) room maintained on a 12-hr light-dark cycle (lights on at 8:00 hr) and provided with food and water ad lib. All exposure sessions were run between 9:00–11:00 hr in the same room.

Procedure of Microwave Irradiation

The 2450-MHz cylindrical waveguide exposure system of Guy *et al.* [6] was used. Four rats were simultaneously subjected to microwave or sham exposure. The exposed rats were irradiated with pulsed (2 μsec pulses, 500 pps), circularly polarized microwaves at a spatially averaged power density of 1 mW/cm^2 within the guide. Control animals were placed in similar waveguides but received no irradiation (sham exposure). The specific absorption rate was determined calorimetrically to be 0.6 W/kg for the size of animals used in our experiments [3]. All experiments were performed blind, i.e., the experimenters doing the irradiation and choline uptake assay did not know whether a certain animal had received microwave or sham irradiation.

Rats were subjected to 10 daily sessions of microwave exposure with 45 min irradiation during each session. Some animals were sham irradiated according to the same schedule. In the 11th session, all rats were randomly assigned to receive a session of either microwave or sham irradiation, and sodium-dependent high-affinity choline uptake was measured in the hippocampus and frontal cortex immediately after exposure. In addition, on the 11th day, choline uptake was measured in some rats without first putting them in the waveguides. The resulting treatment groups and the effects studied are as follows:

Ten-Day Exposure To:	Day 11 Exposure To:	Effect Studied:
1 Microwave	Microwave	Tolerance
2 Microwave	Sham	Conditioned effect
3 Sham	Microwave	Unconditioned effect
4 Sham	Sham	Control
5 Microwave	None	Control
6 Sham	None	Control

In the following discussion, each group will be signified by a testing category. For example, "microwave/microwave" (10-day-exposure condition/testing condition) represents the group of animals subjected to 10 daily sessions of microwave exposure and tested with microwave on day 11.

With experimental group 1 (microwave/microwave) we examined the development of tolerance to the effect of microwaves after repeated exposure. The effect of microwaves should diminish if tolerance develops. We examined the conditioned effect in group 2 (microwave/sham). The hypothesis was that repeated exposure to microwaves would condition the effect of microwaves on choline uptake to environmental cues. Thus, when an animal was tested on day 11 with sham exposure, those cues associated with exposure, including handling and the waveguide (conditioned stimuli), triggered a conditioned effect. The response of group 3 (sham/microwave) tested the unconditioned effect of microwaves. These animals were exposed to microwaves for the first time on day 11. Group 4 (sham/sham) was the control group for the experiment. Rats in groups 5 (microwave/none) and 6 (sham/sham) had choline uptake measured without exposure in waveguides on day 11. Data from these groups provided additional controls for the possible long-term effects of microwave exposure and handling, and gave additional support that cues in the waveguide were the conditioned stimuli.

Method of Sodium-Dependent High-Affinity Choline Uptake Assay

Rats were sacrificed by decapitation and their brains dissected out. The hippocampus and frontal cortex were dissected on ice. The frontal cortex consisted of the cerebral cortex anterior to a coronal cut at the level of the optic chiasma, with the olfactory tubercle, septum, and frontal portion of the striatum removed.

Sodium-dependent high-affinity choline uptake by brain synaptosomes was assayed by a method previously described [25]. Brain tissue was homogenized in 2 ml of 0.32 M sucrose solution using a glass pestle homogenizer. The homogenate was centrifuged at 1,000 × g for 10 min. The supernatant was then recentrifuged at 17,000 × g for 15 min, and the pellet was reconstituted in 2 ml of 0.27 M sucrose. Of this synaptosomal preparation, 0.1 ml was added to each of a set of tubes containing 0.9 ml of a buffer (containing 4% dextrose, 126 mM NaCl, 1.28 mM Na_2HPO_4, 4.75 mM KCl, 1.27 mM $CaCl_2$, and 1.42 mM $MgCl_2$; pH 7.2), 0.3 μM choline chloride, and 0.4 μCi of ^{3}H-choline (80 Ci/mmol, New England Nuclear, Boston, MA). Nonsodium-dependent choline uptake was determined by addition of 0.3 μM of hemicholinium-3 (Sigma Chemicals, St. Louis, MO) to a similar set of tubes. Each brain sample was assayed in triplicate.

The samples were transferred from an ice bath to a water bath at 38°C for incubation for 4 min. Uptake was terminated by return of the samples to the ice bath. Synaptosomes were collected by centrifugation at 8,000 × g for 20 min. The supernatant was discarded, and the pellet was washed with 1 ml of ice-cold 0.9% saline. The saline was removed, and the pellet was dissolved overnight with 0.7 ml of Protosol (New England Nuclear, Boston, MA). Protosol was then neutralized with 30 μl of glacial acetic acid, and 8 ml of Econofluor (New England Nuclear, Boston, MA) was added. Radioactivity was determined by liquid scintillation. High-affinity choline uptake was determined as the difference in uptake in the absence and presence of hemicholinium-3, and was found to comprise approximately 40–50% of the total uptake. Protein concentration of the synaptosomal preparation was determined by the method of Lowry *et al.* [16] using bovine serum albumin as standards.

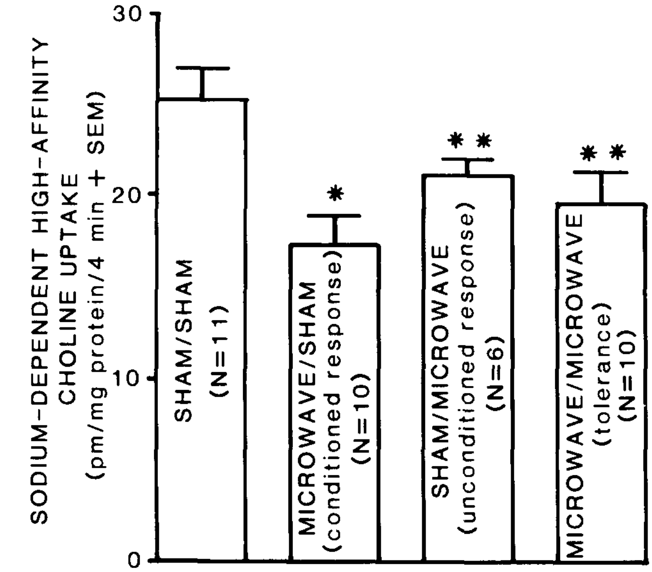

FIG. 3. Choline uptake in the frontal cortex of rats in the different exposure-treatment groups assayed after exposure session 11. One-way ANOVA of the data showed a significant treatment effect: $F(3,33)=4.93$, $p<0.01$. *,**Significantly different from response of the sham/sham rats at $p<0.01$ and 0.05, respectively.

Data Analysis

Sodium-dependent high-affinity choline uptake is expressed as pm/mg protein/4-min. Data with two treatment groups were compared by the 2-tailed Student's *t*-test. Data involving more than two treatment groups were analysed by the one-way analysis of variance, and difference between two treatment groups was compared by the Newman-Keuls test. A difference at $p<0.05$ was considered statistically significant.

RESULTS

Effects of acute microwave exposure on choline uptake in the hippocampus and frontal cortex are shown in Fig. 1. uptake in both brain regions of the microwave-irradiated rats is significantly lower than that of the sham-irradiated animals ($p<0.01$).

Data of repeated exposure on choline uptake in the hippocampus and frontal cortex are shown in Figs. 2 and 3, respectively. There was a significant treatment effect on hippocampal choline uptake, $F(3,32)=9.89$, $p<0.01$ (Fig. 2). There was no significant difference in uptake between the microwave/microwave and sham/sham animals, suggesting development of tolerance after repeated exposure. The conditioned response (microwave/sham) was significantly higher than the response of the sham/sham rats ($p<0.01$). Thus, the effect was opposite to that observed after acute exposure (see Fig. 1). The response of the sham/microwave (unconditioned response) rats was significantly lower than that of the sham/sham animals ($p<0.05$). This effect was similar to that observed in the acute microwave exposed animals.

A significant treatment effect was also found on frontal cortical choline uptake, $F(3,33)=4.93$, $p<0.01$ (Fig. 3). No tolerance developed to the effect of microwaves, i.e., response of the microwave/microwave rats was similar to that of the acute microwave-irradiated rats, and significantly

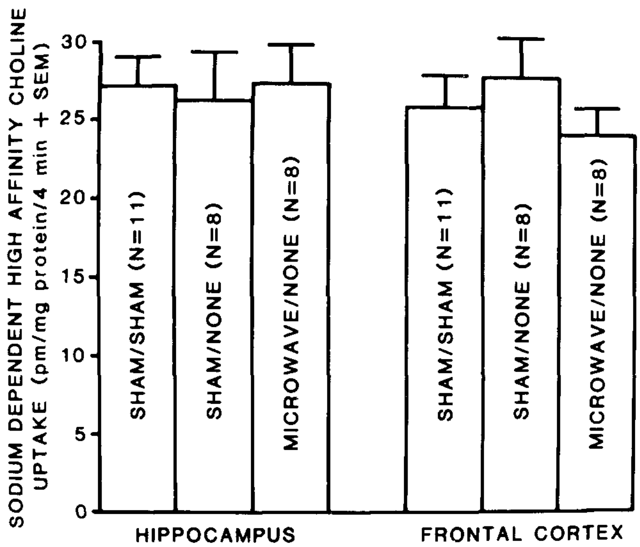

FIG. 4. Choline uptake in the hippocampus and frontal cortex of the sham/sham, sham/none, and microwave/none animals. No significant difference was found among the different treatment groups.

lower than that of the sham/sham rats ($p<0.05$). A conditioned response was seen in the microwave/sham rats that was similar to the unconditioned effect, i.e., a decrease in choline uptake (significantly different from the response of sham/sham animals at $p<0.01$). Furthermore, responses of the sham/microwave rats (unconditioned effect) were significantly smaller than those of the sham/sham rats ($p<0.05$).

Figure 4 shows the data for both hippocampus and frontal cortex of the microwave/none and sham/none animals as compared to the data of the sham/sham animals. In both brain regions, no significant difference was found among these treatment groups.

DISCUSSION

Data presented in this paper further support our previous findings that the effects of low-level pulsed microwave irradiation are classically conditionable. After repeated pairing with microwaves, cues in the exposure environment probably became the conditioned stimuli of the effect and triggered a conditioned response when presented alone. In this respect, the effects of microwaves are similar to those of psychoactive drugs. It is well established that the effects of drugs are classically conditionable to environmental cues [20,24].

In previous research, we found that the effect of microwaves on amphetamine-hyperthermia [13] and ethanol-hypothermia [11] are classically conditionable. In addition, we found that a transient hyperthermia seen immediately after acute exposure to microwaves could also be classically conditioned to environmental cues [12]. These phenomena probably involve different neural mechanisms. Thus, these data would suggest that a variety of neural functions affected by microwaves are classically conditionable. It is not known how low-level microwave irradiation exerts its effects on the nervous system. Better understanding of the conditioned effects will only come when the primary neural mechanisms affected by microwaves are identified. Possible candidates are the thermoregulatory mechanism [1] and the auditory system [2]. Both of these systems are known to be affected by pulsed microwave radiation. Particularly, disturbance of thermoregulatory functions may lead to change in hippocampal cholinergic activity, since rats under irradiation are actively dissipating the heat load from the low-level microwaves [9]. We have recently reported that activity of the hippocampal, but not the cortical, cholinergic pathway is dependent on body temperature [23]. Furthermore, thermoresponsive neurons have also been reported in the ventral septal area [4], where some of the septo-hippocampal cholinergic cell bodies are located.

It is interesting that the conditioned effects on the hippocampus and frontal cortex are different. For the hippocampus, the conditioned effect was opposite to the unconditioned effect of microwaves, whereas for the frontal cortex, the conditioned and unconditioned effects were similar. Both types of conditioned effects, i.e., similar or opposite in direction to the unconditioned effects, have been reported in the literature [17]. The cholinergic innervations to the hippocampus and frontal cortex come from different origins, i.e., the medial septum and nucleus basalis magnocellularis, respectively. The pharmacological and neurological properties of these two pathways are different [18] and their responses to classical conditioning are probably different. Such differences could also account for the finding that tolerance developed to the effects of microwaves in the hippocampus but not in the frontal cortex after 10 sessions of exposure.

In a review paper [15], we compared the biological effects of low-level microwaves and of stressors and concluded that they have similar patterns and that stress may be a mediating factor of microwave irradiation. Recently, we have reported that restraint-stress exerts effects similar to microwaves on choline uptake in the hippocampus and frontal cortex [7,14]. Decreases in choline uptake were observed in both cases. Furthermore, in both instances, the effect on hippocampal choline uptake was blocked by treatment with narcotic antagonists, whereas the effect on the frontal cortex was not significantly affected.

Studies on the responses of the hippocampal cholinergic system to restraint stress have shown that the response is biphasic, i.e., an excitation phase followed by a depression phase [5]. Measurement of choline uptake in the hippocampus of rats subjected to restraint showed that uptake activity was increased after a short period and decreased after a longer period of restraint. The latter phase was explained as a result of exhaustion after the initial excitation phase. Perhaps this biphasic response, in addition to being a function of time, is also a function of the intensity of the stressor. Uptake increases after exposure to a stressor at low intensity, but decreases after exposure to the stressor at higher intensity for the same period of time. This biphasic response may explain the difference in choline uptake in the hippocampus for the unconditioned and conditioned responses to microwaves. The effect of direct microwave irradiation is intensive enough to cause a decrease in choline uptake, whereas the conditioned effect is less intense and causes an increase in uptake. Response characteristics of the cholinergic system in the frontal cortex have not been very well studied. Perhaps, the depression phase predominates and decrease in choline uptake is observed over a wide range of intensities of the stressor. Thus, responses to both conditioned and unconditioned effects of microwaves are similar. Further studies varying the intensity and duration of microwave exposure will be necessary.

Central cholinergic systems are involved in a variety of behavioral and physiological functions [22]. Effects of acute low-level microwave irradiation on cholinergic systems may have detrimental consequences for the animals and are thus worth studying.

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