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# Influence of Microwave Exposure on Chlordiazepoxide Effects in the Mouse Staircase Test

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QUOCK, R. M., B. J. KLAUENBERG, W. D. HURT AND J. H. MERRITT. *Influence of microwave exposure on chlordiazepoxide effects in the mouse staircase test.* PHARMACOL BIOCHEM BEHAV 47(4) 845–849, 1994.—To ascertain whether behavioral effects of benzodiazepines are altered by exposure to microwave radiation, we compared the performance of male, Swiss CD1 mice in the staircase test 30 min after pretreatment with chlordiazepoxide (8, 16, and 32 mg/kg, IP) and immediately following a 5-min exposure to microwave radiation (4, 12, and 36 W/kg, continuous wave, 1.8 or 4.7 GHz). In this paradigm, chlordiazepoxide reduction in the number of rears (NR) and number of steps ascended (NSA) is postulated to reflect anxiolytic and sedative drug effects, respectively. In sham-exposed mice, increasing doses of chlordiazepoxide increased NSA without affecting NR, increased NSA and decreased NR, then decreased both NSA and NR. Microwave exposure generally did not alter NSA or NR in mice pretreated with lower doses of chlordiazepoxide. However, in mice pretreated with 32 mg/kg chlordiazepoxide, exposure to 36 W/kg microwave radiation significantly reversed the reductions in NSA and NR at 4.7 GHz but not at 1.8 GHz. These findings indicate that exposure to microwave radiation can selectively alter effects of chlordiazepoxide in this psychopharmacological paradigm.

Microwave exposure  Chlordiazepoxide  Mouse staircase test

THE last decade has witnessed marked proliferation in the use of microwave-emitters in radar equipment, air traffic control systems, satellite and long distance communication networks, and microwave ovens. Such widespread usage of microwave-emitting equipment in the general public, industrial, and military sectors has generated concern among some scientists and some public health officials regarding possible harmful effects of acute or chronic exposure to microwaves (7).

Benzodiazepine drugs are widely used in medicine for their sedative/hypnotic, skeletal muscle relaxant, anxiolytic, and anticonvulsant properties (13). Considering the marked increase in use of microwave-emitting equipment, it is conceivable that persons who use such medications might be exposed to radiofrequency electromagnetic fields. Research on the influence of microwave irradiation on benzodiazepine drug effects has been limited and their results inconsistent. Exposure to various frequencies of microwaves has been reported to enhance certain effects of benzodiazepines (5,18), reverse other effects of benzodiazepines (4,8), and have no effect on still other effects of benzodiazepines (8,14–16,19). These findings reveal that the interaction between microwaves and benzodiazepines to be very diverse and complex.

The mouse staircase test is a simple and efficient test for preliminary assessment of anxiolytic and sedative effects of benzodiazepines (17), and we have enjoyed success in employing it to investigate actions and interactions of benzodiazepine and other anxiolytic drugs (4,9–12,20). In this particular paradigm, step-climbing is thought to be indicative of locomotor activity, and drug suppression of step-climbing reflects a sedative drug effect. On the other hand, rearing behavior is purported to reflect anxiety, and drug suppression of rearing reflects an antianxiety drug effect. In this paradigm, benzodiazepines in increasing doses will decrease NR with no change or even an increase in NSA, although, at higher doses, both NR and NSA will be equally suppressed (4). We will demonstrate herein that exposure to microwave radiation, at levels

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which alone do not alter NR or NSA at the frequencies tested, can, nonetheless, selectively alter behavioral effects of the benzodiazepine chlordiazepoxide in the mouse staircase paradigm.

## METHOD

### *Animals*

Male Swiss CD1 mice, weighing 25–30 g, were purchased from Charles River Laboratories (Portage, MI) and housed in groups of five per cage in standard polycarbonate cages (24 cm L × 14 cm W × 13 cm H) under controlled temperature and humidity. Because a lack of familiarity with the staircase environment is required for production of anxiety in this paradigm, animals were used only once. Animals were treated, exposed, and tested according to the time schedule shown in Fig. 1.

### *Behavioral Testing*

Drug- and vehicle-pretreated mice were subjected to microwave radiation or sham exposure then placed into a staircase constructed of polyvinyl chloride (PVC) or Styrofoam, according to specifications (17). A Panasonic 5000 videocamera and a Panasonic AG-1950 recorder were used to record the NR and NSA for each mouse during a 3-min observation period. The videotapes were later reviewed and NR and NSA for each individual mouse determined by an observer without knowledge of the treatment of each animal. A rear was registered when a mouse stood on its hindlegs, away from or up against a wall. A step was registered when a mouse completely ascended a step, placing all four paws on the step. Only steps ascended were counted; steps descended were not counted to simplify counting. At the end of the 3-min observation period, the animal was returned to its cage and the staircase was cleaned with a wet sponge to remove residual odors.

### *Exposure to Microwave Radiation*

Mice were individually placed inside Plexiglas cylindrical restrainers. The restrainers were placed on a table in front of the pyramidal horn antenna of a Cober Model 1831 Microwave Transmitter inside an anechoic chamber. The animals were exposed in E-polarization at distances of 200 and 146 cm from the standard gain horn for frequencies of 1.8 and 4.7 GHz, respectively. Mice were irradiated in pairs at specific absorption rates (SARs) of 4, 12, and 36 W/kg at a frequency of either 1.8 or 4.7 GHz. Sham-irradiated mice were similarly restrained and placed inside the anechoic chamber out of the electromagnetic field.

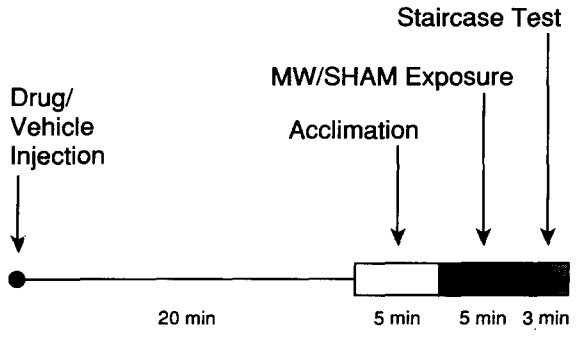


FIG. 1. Time schedule of drug/vehicle treatment, microwave/sham exposure and testing.

### *Determination of SAR*

Dewar-flask calorimetry was used to measure the whole-body average SAR in mice exposed to far-field, continuous-wave RF fields (1,2). For 1.8 GHz, 400 W exposures, the average SAR was 29.2 ± 2.0 W/kg for an average specimen mass of 29.9 ± 1.1 g. For 4.7 GHz, 700 W irradiations, the average SAR was 54.4 ± 3.8 W/kg for an average specimen mass of 27.1 ± 1.3 g.

### *Drugs*

Chlordiazepoxide hydrochloride (Sigma, St. Louis, MO) was dissolved in 0.9% saline and administered by intraperitoneal injection in a volume of 0.1 ml/10 g body weight. Control animals received an intraperitoneal injection of the same volume of vehicle, 0.9% saline.

### *Statistical Analysis of Data*

The mean NR and NSA for each treatment/exposure group were compared, using a three-way analysis of variance (ANOVA) and Tukey's Studentized range test. The significance level for statistical analysis was set at 0.05.

## RESULTS

In sham-irradiated mice, vehicle pretreatment generally resulted in 20–25 NR and NSA. Increasing doses of chlordiazepoxide sequentially increased NSA without affecting NR (8 mg/kg), then increased NSA while decreasing NR (16 mg/kg), and finally decreased both NSA and NR (32 mg/kg).

At 1.8 GHz (Figs. 2A and 2C), increasing SARs of 4, 12, and 36 W/kg generally did not significantly influence NR or NSA of mice pretreated with vehicle or 8 or 16 mg/kg chlordiazepoxide. However, mice pretreated with 32 mg/kg chlordiazepoxide exhibited significantly greater NSA at SARs 12 and 36 W/kg and significantly greater NR at SAR 36 W/kg.

At 4.7 GHz (Figs. 2B and 2D), increasing SARs of 4, 12, and 36 W/kg generally did not significantly influence NR or NSA of mice pretreated with vehicle or 8 or 16 mg/kg chlordiazepoxide. However, mice pretreated with the greatest dose of chlordiazepoxide (32 mg/kg) exhibited significantly greater NSA at all SARs and significantly greater NR at SAR 36 W/kg. These were the interactions that displayed the largest significant differences and were most likely due to microwave-induced deviation from the generally inverted U-shaped dose-response curve of the sham-exposed chlordiazepoxide control groups.

Statistical analyses of these findings indicate the following:

1. Relative to NR,
   a. There was a significant overall effect of drug dose, $F(3, 320) = 82.55$, $p < 0.0001$. Tukey's post hoc comparisons showed that chlordiazepoxide doses 16 and 32 but not 8 mg/kg significantly lowered NR, compared to the vehicle group; in addition, chlordiazepoxide dose 32 mg/kg reduced NR significantly more than either 8 or 16 mg/kg, and 16 mg/kg reduced NR significantly more than 8 mg/kg.
   b. There was a significant overall effect of SAR, $F(3, 320) = 4.18$, $p < 0.006$. The effect of an SAR of 36 W/kg on NR was significantly greater than that of 4 and 12 W/kg as well as the sham-exposed group. The effects of SAR 4 and 12 W/kg were not significantly different

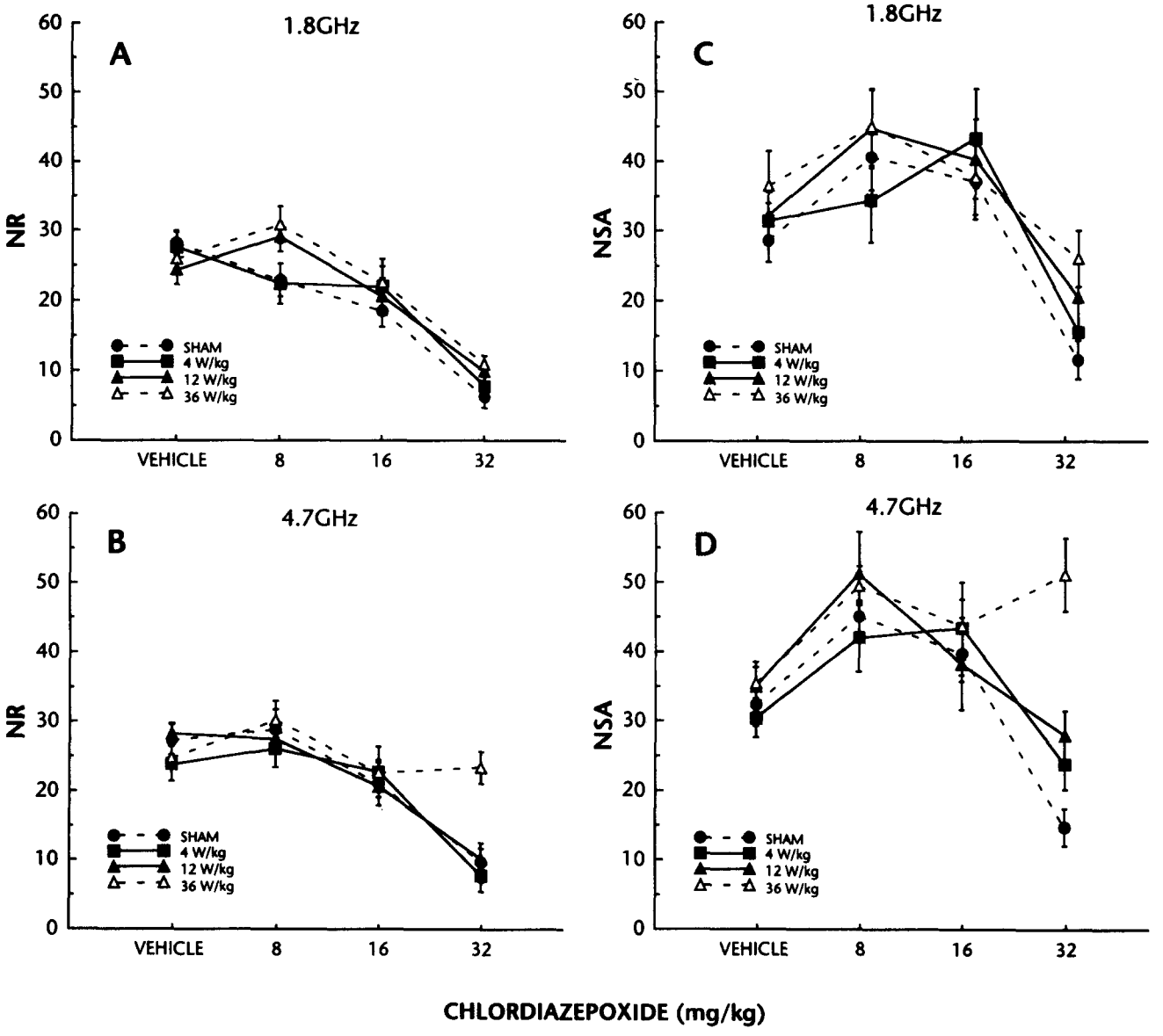


FIG. 2. Effects of drug treatment (vehicle and three doses of chlordiazepoxide) and microwave exposure (sham and three SARs) on NR at 1.8 GHz (A) and 4.7 GHz (B) and on NSA at 1.8 GHz (C) and 4.7 GHz (D).

from that of the sham group. There was also no significant difference between NR effects of SARs 4 and 12 W/kg.

c. Although there was no significant overall effect of frequency, $F(1, 320) = 3.40$, $p > 0.06$, analyses did yield a significant interaction between drug dose and SAR, $F(9, 320) = 1.98$, $p < 0.05$. Reference to Fig. 2B suggests that the statistical interaction is due to the lack of a chlordiazepoxide dose-related decrease in NR in the 32 mg/kg-36 W/kg group at 4.7 GHz.

2. Relative to effects on NSA,
   a. There was a significant overall effect of drug dose, $F(3, 320) = 30.02$, $p < 0.0001$. Chlordiazepoxide doses 8 and 16 mg/kg both elevated NSA, while 32 mg/kg significantly depressed NSA, compared to the vehicle group. The NSA effect of the group treated with 32 mg/kg was also significantly less than that of groups treated with 8 and 16 mg/kg. There was no significant difference in NSA between doses 8 and 16 mg/kg.
   b. There was a significant overall effect of SAR, $F(3, 320) = 5.95$, $p < 0.0006$. There were no significant differences in NSA between sham and either SARs 4 and 12 W/kg. The mean NSA at SAR 36 W/kg was significantly greater than that of the sham group and SAR 4 W/kg but not SAR 12 W/kg.
   c. There was a significant overall effect of frequency, $F(1, 320) = 9.63$, $p < 0.002$. Reference to Figs. 2C and 2D shows that the largest difference due to microwave frequency occurred in the 32 mg/kg-36 W/kg group. SAR 36 W/kg at 4.7 GHz but not 1.8 GHz blocked the decrease in NR and NSA that was observed in groups treated with 32 mg/kg.
   d. There was also a significant effect of drug dose × SAR, $F(9, 320) = 2.30$, $p < 0.016$.

## DISCUSSION

Earlier studies have produced equivocal findings on the influence of microwave irradiation on various drug effects of benzodiazepines. Acute exposure (30 min) to pulsed microwaves (2 $\mu$s, 500 pps, 2.45 GHz, average power density = 1.0 mW/cm$^2$) reportedly potentiated the response-rate increasing effect of chlordiazepoxide in rats in operant behavioral tests; exposure to such low intensity microwave radiation alone had no influence upon this behavior of rats (16,18). However, this potentiating effect on benzodiazepines was not observed following exposure to 915 MHz (15) or 2.8 GHz microwave fields (14,19). The inconsistencies in these studies might be due to differences in electromagnetic frequency, cumulative effects of repeated exposures, or even a conditioning effect of microwave exposure.

In other studies, repeated (21 × 45-min sessions) exposure of rats to pulsed (2 $\mu$s, 500 pps, 2.45 GHz, average SAR = 0.6 W/kg) microwaves was found to reduce the sedative effect of diazepam while increasing its appetite-suppressant effect (5). Exposure to a pulsed microwave field (2.0 $\mu$s, 500 pps, 2.7 GHz, average SAR = 0.75–3.0 mW/cm$^2$) at power densities up to 20 mW/cm$^2$ also counteracted the hypothermic effect of chlordiazepoxide without affecting its ability to suppress pentylenetetrazol-induced seizures in rats (8). These findings suggest that microwave irradiation might influence some effects of benzodiazepines (such as sedative, thermotropic, and ingestive effects) but not others (anticonvulsant).

In the present investigation, we assessed the interaction between microwave radiation and chlordiazepoxide in the mouse staircase test, a paradigm that screens for anxiolytic drug activity (17). The doses of chlordiazepoxide used were derived from an earlier study in which we characterized the dose-response relationship for chlordiazepoxide in this test (12). These doses progressively suppressed NR while causing first increased, then unchanged, and finally decreased NSA, respectively, with increasing dose. The microwave exposure levels were much higher than those allowed by safety standards and were not comparable to those encountered by the public or found in occupational situations.

Our results show that, when microwaves are superimposed on chlordiazepoxide treatment, we found that SAR 36 W/kg antagonized or even reversed the ability of high doses of chlordiazepoxide to suppress NSA. The data also indicate that microwave exposure usually failed to alter the ability of chlordiazepoxide to suppress NR, the one exception being the effect of SAR 36 W/kg on 32 mg/kg of chlordiazepoxide. These findings, if interpreted in strict accordance with the premise of the staircase test (4,17), suggest that microwave exposure generally does not interfere with the anxiolytic effect of benzodiazepines, while possibly antagonizing the sedative side effect of benzodiazepines. These results would be in agreement with earlier studies that microwave irradiation can antagonize the sedative but usually not the operant behavioral effects of benzodiazepines.

Our results demonstrate an antagonism by microwave exposure of chlordiazepoxide sedation at both 1.8 and 4.7 GHz and of chlordiazepoxide anxiolysis at 4.7 GHz only. The greater interaction of microwave exposure with chlordiazepoxide at 4.7 GHz can be attributed to the fact that the resonance frequency for a mouse is approximately 1.8 GHz (3). The increased peripheral deposition of energy at the nonresonant frequency is more likely to induce stress and counteract the anxiolytic effect of chlordiazepoxide.

One possible explanation for how microwave exposure might reverse the chlordiazepoxide effect is that acute microwave exposure might influence benzodiazepine receptors and thereby alter responsiveness to benzodiazepine drugs. However, Lai et al. (6) recently reported increased concentration but unchanged affinity of [$^3$H]flunitrazepam binding sites in the cerebral cortex of rats following a single 45-min exposure to pulsed microwaves (2 $\mu$s, 500 pps, 2.45 GHz, average SAR = 0.6 W/kg). This observation would indicate that there is no downregulation of benzodiazepine receptors following acute microwave exposure. Therefore, a mechanism for the microwave–benzodiazepine interactions observed in the present study may lie at some level other than the benzodiazepine receptor.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources--National Research Council. The Armstrong Laboratory has been fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) since 1967.

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