Microwave Effect on Embryo Brain - Dose Dependence and the Effect of Modulation

Yu. Grigoriev, V. Stepanov
Center of Electromagnetic Safety, The State Research Center of Russia –
Institute of Biophysics,
123182 Moscow, Russia, Zhivopisnaya,46

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Introduction

There are many publications showing that the nervous system is especially sensitive of to EMF. We suppose that EMF effects on the nervous system are critical for the evaluation of possible health hazards from EMF exposure. Earlier we demonstrated that imprinting is a good model for studying effects of low levels of various physical factors on the embryonic nervous system. We are not aware of any studies of EMF effects on imprinting behavior.

The goals of this study were: (1) to develop response spectra for CW and pulse-modulated microwaves with respect to the field intensity, and to determine threshold intensities, and (2) to investigate the principle possibility of detection and "memorizing of a modulated microwave signal by the brain of chick embryo.

Material and methods

Experiments with imprinting were carried out on chicks; a total of 447 chick embryos were used. The embryos were incubated at 37.5-38.0 °C, 55-68% relative humidity for 21-22 days. The embryos were subjected to a short-term exposure to 10-GHz microwaves at various periods of the incubation. Exposures were performed in an anechoic chamber. Parallel controls to each set of experiments were handled in the same way, but received sham irradiation. Tested levels of the incident power density were 0.04, 0.4, 1.0, 8.0, and 10 mW/cm^2.

An imprint-stimulus (either a moving object or light flashes) was presented the first time during the receptive period, 24 hours after hatching. The imprinting behavior was evaluated 24 hours later, i.e., 48 hours after hatching.

Quantitative evaluation of the imprinting behavior was based on the following parameters: latency of the response to the imprint-stimulus, the duration of time interval when the chick remained near the imprint-stimulus, and the number of approaches to and contacts with the stimulus.

A double-blind test procedure was used. Results were statistically evaluated by Student's and Fisher's parametric tests and by a non-parametric Vilkison criterion.

Results

EMF effect on memory formation during the early postnatal period. In this first series, we performed three sets of experiments. Embryos were exposed a single time, on the 5th, 16th, or 19th day of incubation (Table 1). The exposure lasted for 30 min at 0.4, 1, 8, or 10 mW/cm^2.

Table 1. Conditions of experiments for Series 1.

Set number	Incubation day+ EMF exposure	Frequency	EMF Parameters: Conditions of exposure	EMF Parameters: Power density, mW/cm^2	EMF Parameters: exposure duration, min	Number of embryos: control	Number of embryos: experiment
I	5th	10 GHz	CW	1, 8 and 10	30	27	22
II	16th	10 GHz	CW	1	30	9	10
III	19th	10 GHz	CW	0.4	30	18	18

Results of the Set 1 are summarized in Table 2. In subsets 1.1 and 1.2, the imprinting behavior was produced in 100% of control chicks, but in none of those exposed at 10 or 8 mW/cm^2. Thus, exposure of embryos at these power densities completely suppressed the ability of newborn chicks to develop imprinting. Exposure at a lower power density of 1 mW/cm^2 had a less pronounced effect: the imprinting response was produced in 9 out of 10 controls, and in 4 out of 7 exposed chicks.

Table 2 . Imprinting behavior in chicks that were exposed in egg on the 5th day of incubation.

Subsets	Power density, mW/cm^2	Exposure duration, min	Groups	Number of chicks	Number of chick with imprinting
1.1	10	-	control	6	6
		30	experiment	7	0
1.2	8	-	control	11	11
		30	experiment	8	0
1.3	1	-	control	10	9
		30	experiment	7	4

In set 2, chick embryos were exposed on the 16th day of incubation. The results were similar to those in subset 1.3. Eight out of 9 chicks developed imprinting in the control group, and 6 out of 10 in the exposed group. Apparently, the day of exposure was not as significant as the incident power density. This allowed us to pool together all the data for exposures at 1 mW/cm^2 (i.e., from the subset 1.3 and set 2). In this combined group, the imprinting behavior developed in 17 out of 19 controls and in chicks, and in 10 out 17 exposed at 1 mW/cm^2 ($p < 0.05$).

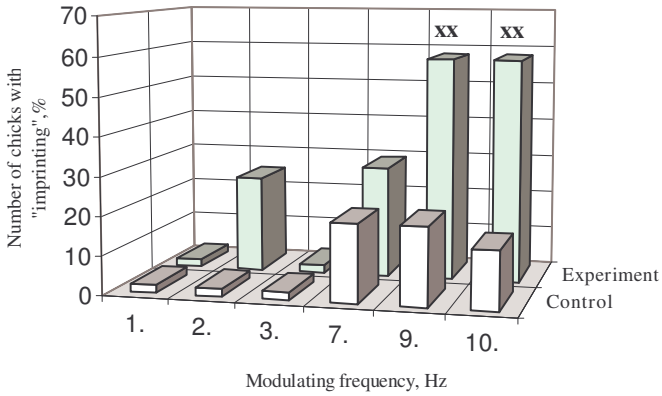
In set III, embryos were exposed on the 19th day of incubation at the power density of 0.4 mW/cm^2. The imprinting behavior was established in 17 out of 18 controls, and in 13 out of 18 exposed chicks. This difference was not statistically significant ($p < 0.1$). However, the data combined for all the 3 groups exposed at low intensities (0.4 and 1 mW/cm^2) showed a highly significant effect (34 chicks with imprinting out 37 in controls versus 23 out of 35 in the exposed groups, $p < 0.01$).

Therefore, we conclude that the power densities of 8 and 10 mW/cm^2 produce a strong effect on the development of the embryonic nervous system. A manifestation of this effect is the inability of newborn chicks to develop imprinting behavior. We also infer that lower EMF intensities of 0.4 and 1 mW/cm^2 can alter the formation of imprinting behavior in some individuals.

"Memorizing" of the microwave signal by embryo brain. This set of experiments was performed on 127 embryos. On the 16th day of incubation, the embryos were exposed to 10 GHz, 0.04 mW/cm^2 microwaves modulated at 1, 2, 3, 7, 9 and 10 Hz. The exposure duration was 5 min.

We supposed that microwave modulation frequency could be "memorized" by brain, so that after hatching the exposed chicks would prefer light flashes of the same frequency.

Figure 1 Number of embryos prefering EM modulated signal



The imprinting behavior (actually, in this case it was a "preference" behavior) was tested 48 hours after hatching. Chicks had to choose between two stimuli. One stimulus was light flashes with the same frequency as had been used for modulation during the microwave exposure. The other stimulus was the same flashes, but at a different frequency. The difference in the frequencies of flashes of these two stimuli was always 8 Hz. For example, if one frequency was 10 Hz, the other light flashed at 2 Hz; if one frequency was 5 Hz, the other stimulus was set at 13 Hz, and so forth. Indeed, chicks showed preference to the imprint stimulus of 9 or 10 Hz if the embryos had been exposed to EMF with 9 or 10 Hz modulation (Fig. 1). However, exposure at other modulation frequencies (1, 2, 3, or 7 Hz) was not effective.

Influence of modulated microwaves on imprinting behavior. The above data showed that, for some reasons, the embryonic brain was more susceptive to microwave modulation frequencies of 9 and 10 Hz. Therefore, we decided to compare the efficacy of CW 10-Hz modulated irradiation regimens. The endpoint was formation of the imprinting behavior (same procedures as in the series 1). The light flashes, which were used as an imprint-stimulus, were also delivered at a 10-Hz rate.

A total of 129 embryos were exposed or sham-exposed on the 16th day of incubation, and the imprinting behavior was assessed 48 hours after hatching. In this series, we anticipated stronger effects; therefore, the incident power density was decreased to 40 uW/cm^2, and the exposure duration was shortened to 5 min. Results of these experiments are presented in Table 3.

Table 3. Imprinting behavior in chicks after exposure of embryos at 40 uW/cm^2 (10 GHz) on the 16th day of incubation.

Set Number	Power density, uW/cm^2	Exposure duration, min	Exposure regimen	Number of embryos	Number of chicks with imprinting
1.	-	-	Sham	83	81 (97%)
2.	40	5	cw	27	23 (89%)
3.	40	5	10-Hz modulation	19	9 (47%)

One can see from the table that CW irradiation at 40 uW/cm^2 did not produce any changes compared with sham controls. In contrast, the difference between sets 3 and 1 was statistically significant ($p<0.01$). Exposure to 40 uW/cm^2, 10-Hz modulated microwaves prevented formation of imprinting behavior in about 50% of cases.

Thus, a 5-min CW microwave exposure at 40 uW/cm^2 on the 16th day of incubation did not influence formation of the imprinting behavior. The same exposure, but with 10-Hz modulation, caused statistically significant bioeffects, namely it suppressed the ability of chicks to develop imprinting behavior. The data show that, under given experimental conditions, 10-Hz pulse modulation was more effective than the cw exposure regimen.

It was important to find out if using the same frequency for microwave modulation and for the imprint stimulus was a necessary condition for this effect. In the next set, we used microwaves modulated at 10 Hz, but the frequency of light flashes was 2 Hz. The exposure effect was still present and even more profound: The imprinting behavior developed in 6 out of 8 control chicks (75%), but only in 1 out of 8 exposed chicks (15%, $p <0.05$). Thus, keeping the imprint-stimulus frequency the same as the modulation frequency was not essential; exposure to 10-Hz modulated microwaves suppresses imprinting of both 10- and 2-Hz light flashes.

We also carried out a trial experiment using a 40-Hz modulation of microwaves. Out of 8 chicks that were exposed on the 16th day of egg incubation (5 min at 40 uW/cm^2), the imprinting behavior developed in 5 (62%). Because of the small experimental group, this result was not significantly different from the control.

As a next step, we studied how the effect of 10-Hz modulated microwaves depends on the incident power density. The experimental data demonstrated a distinct dose response (Table 4).

Table 4. Imprinting in chicks exposed in egg on the 16th day of incubation to 10-Hz modulated microwaves at different incident power densities.

Sets	Exposure regimen	Power density, uW/cm^2	Exposure duration, min	Number of chick	Number of chicks with imprinting
I	Sham	-	-	28	22 (78%)
II	CW	40	5	10	8 (80%)
III	Modulated 10 Hz	40	5	12	8 (65%)
IV	Modulated 10 Hz	900	5	7	4 (57%)
V	Modulated 10 Hz	1800	5	6	1 (17%)

We consider it useful to summarize the results of all experiments with exposure at 40 uW/cm^2 in a separate table (Table 5). The experiments presented in this table were performed in different animal groups, during different seasons of the year, and also using somewhat different methods of analysis of imprinting behavior.

Table 5. Effect of a 5-min exposure of eggs to 40 uW/cm^2, 10 GHz microwaves on imprinting behavior in newborn chicks.

Modulation	Power density, uW/cm^2	Number of embryos	Number of chicks with imprinting
Sham-exposed			
		36	35 (97%)
		8	8 (100%)
		9	8 (89%)
		28	22 (78%)
		10	8 (80%)
		8	6 (75%)
CW			
	40	18	16 (89%)
		9	7 (78%)
		10	8 (80%)
Modulation			
10 Hz	40	8	3 (37%)
		11	6 (64%)
		12	8 (65%)
10 Hz	40	8	1 (12%) imprint-stimulus - 2Hz
40 Hz	40	8	5 (62%)

The results presented in Table 5 show that intensity of 40 uW/cm^2 may be subthreshold for CW exposure. However, at 10-Hz modulation, even a 5-min exposure is sufficient to produce changes in imprinting behavior.

Conclusions

1. The influence of a short-term exposure to 10-GHz microwaves on imprinting behavior was studied in 447 newborn chicks. CW and pulse-modulated regimens at intensities from 40 uW/cm^2 to 10 mW/cm^2 were tested.
2. The experiments established that a single short-term exposure to CW or pulse-modulated microwaves at the average incident power densities from 0.4 to 10 mW/cm^2 suppressed the imprinting behavior.
3. The effect of exposure on the imprinting behavior alterations showed a clear correlation with the field intensity employed.
4. Microwaves modulated at 10 Hz were more effective than CW radiation at the same incident power density.
5. The intensity of 40 uW/cm^2 was subthreshold to alter the imprinting behaviour in chicks by a short-term CW irradiation of embryos. However, the threshold for modulated microwaves was below 40 uW/cm^2.
6. It was also demonstrated that a low-intensity modulated microwave signal can be detected and "memorized" by the embryo brain.

These new findings should be taken into account for assessment of possible health hazards from exposure to modulated microwaves.