

# Broadband, Non-Thermal Millimeter-Wave Influence on Giant Chromosomes

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Non-Thermal, Broadband Millimeter-Wave Effect, Putting in Giant Chromosomes, Coherence

A non-thermal influence of low-intensity millimeter-wave radiation on the puffing of giant chromosomes from salivary glands of larvae of the midge *Acricotopus lucidus* (Diptera, Chironomidae) is reported. The effect is manifested as a strong regression in size of a specific puff that expresses genes for a secretory protein (Fig. 1). While millimeter-wave irradiation leads to an about tenfold increase of the regression probability compared to controls, simulation of the small microwave-induced temperature increase in the sample does not result in a significant effect, thus confirming the non-thermal nature of the microwave irradiation effect. This finding could be of importance for the understanding of the interaction between microwave radiation and living systems and hence for the establishment of safety standards in that frequency regime.

Non-thermal biological effects [1–6] of low-intensity microwave radiation, *i.e.* biological microwave effects which cannot be explained as caused by heating, are still a controversial topic in the scientific discussion [7]. In this paper we confirm our previous observation [6] of the influence of low-intensity millimeter-wave radiation on the puffing of giant chromosomes. Using improved experimentation we show that millimeter-wave irradiation causes an about tenfold increase of the regression probability of a certain Balbianiring in giant chromosomes, far beyond any statistical uncertainty. Extending the frequency range we find the effect to be broadband in frequency from 40 GHz (1 GHz =  $10^9$  Hz) to 80 GHz.

The millimeter-wave irradiation system (Fig. 2) included two electromagnetically isolated chambers; one for irradiation and one for control. Fused silica sample containers (s. Fig. 2) were used to keep the salivary glands (diameter: 0.3 mm) in Cannon's Medium [8] at a defined position in the microwave field. The containers were covered by an oxygen permeable membrane to guarantee gas exchange and were positioned on a fused-silica temperature-controlled dish ( $9.0 \pm 0.2$  °C). The microwave-induced temperature increase was measured with a micro-miniature thermal probe and was found to be less than 0.3 °C at 20 mW forward power.

Different microwave frequencies between 40 GHz and 80 GHz were arbitrarily chosen (41.200 GHz,



Fig. 1. a) Balbianiring BR 2 (control) fully decondensed. b) BR 2 locus after 2 hrs irradiation with millimeter-waves. The BR 2 (s. arrow) has regressed. The chromatin fibrils are totally condensed and the surrounding puff-material (ribonucleoprotein) has disappeared. Scale bars: 10  $\mu$ m.

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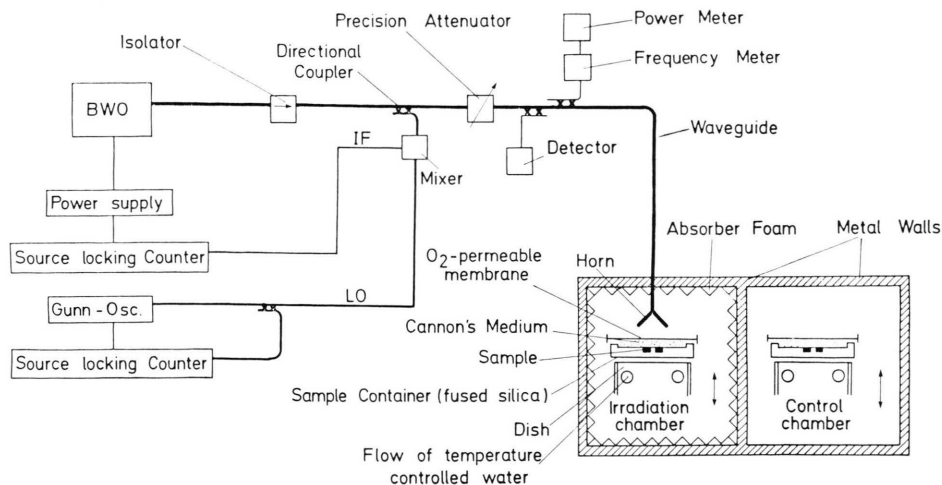


Fig. 2. Schematic description of the millimeter-wave irradiation system. The sample container consists of a fused silica plate with an indented circle of radius 40 mm and depth  $0.2 \pm 0.02$  mm. In its centre two further circular incisions of diameter 2 mm and depth  $0.3 \pm 0.02$  mm have been prepared. One salivary gland is put in each of these last indentations and Cannon's medium (8) is added.

45.200 GHz, 67.200 GHz, 68.200 GHz, 80.200 GHz). By use of a phase-locking loop the frequencies were stabilized with an accuracy of  $\pm 0.5$  MHz. The forward power was measured as  $20 \pm 0.5$  mW and the power reflected by the sample container as  $2 \pm 0.5$  mW. Thus a power of  $18 \pm 1$  mW entered the sample container. Considering the attenuation in the medium of the salivary glands (thickness of the medium layer was at least 200  $\mu$ m), a power flux density of less than 6 mW/cm<sup>2</sup> results (horn area 1.6 cm<sup>2</sup>).

For the experiment the paired salivary glands from larvae of the fourth larval instar of *Acricotopus lucidus* were dissected. One gland was placed in the irradiation chamber and the other in the control chamber (s. Fig. 2). Each gland is composed of two clearly differentiated cell-types. These cell-types correspond to morphologically distinct lobes which are characterized by a specific pattern of predominant puffs (Balbianirings) [9–13] and are designated main lobe and anterior lobe. Each main lobe consists of about 50–60 cells whereas the smaller anterior lobe has 12–20 cells. The nuclei of all cells contain three polytene chromosomes. In the cells of the main lobe two cell-type specific Balbiani rings (BR 1 and BR 2) are developed in chromosomes I and II, respectively. Only the BR 2 was microscopically examined because in preceding experiments no clear reactions at other Balbianiring sites had been observed.

Immediately after exposure (2 hrs) each gland was fixed with ethanol-acetic acid (3:1). The samples were stained for squash preparations. Three classes of BR 2 sizes were distinguished: normal (diameter greater than fourfold chromosome diameter), weakly reduced (diameter between twofold and fourfold chromosome diameter) and strongly reduced (diameter less than twofold chromosome diameter). For each gland the number of normal ( $n_1$ ), weakly reduced ( $n_2$ ) and strongly reduced ( $n_3$ ) Balbianirings BR 2 was determined and the percentage probability of strongly reduced Balbianirings BR 2  $r = (n_3 / (n_1 + n_2 + n_3)) \cdot 100$  was calculated for the sample in the irradiation chamber ( $r^{\text{irr.}}$ ) and in the control chamber ( $r^{\text{contr.}}$ ). All experiments were carried out blind, i.e. the examining biologist did not know if a sample had been placed in the irradiation- or in the control-chamber.

For the sham-irradiated samples no significant difference could be detected between samples placed in the irradiation- and the control-chamber (Fig. 3, Table I). In contrast to that, the millimeter-wave irradiated samples showed an about tenfold increase of the regression probability  $r$  of the BR 2 compared to controls. The effect is highly significant as proven by the U-Test of Mann-Whitney which is non-parametric [14].

To check if the observed effects were caused by the microwave-induced temperature changes the

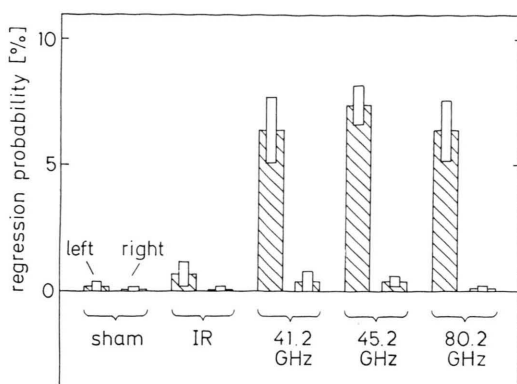


Fig. 3. Percentage regression probability  $r^{\text{irr.}}$  for the left (irradiation) chamber and  $r^{\text{contr.}}$  for the right (control) chamber. The shaded area describes the mean, the open area the standard error of the mean, "sham" stands for sham-irradiation, "IR" for irradiation with infrared light of  $20 \mu\text{m} \leq \lambda \leq 70 \mu\text{m}$ . 41.2 GHz, 45.2 GHz and 80.2 GHz is the frequency of the millimeter-wave irradiation; power density  $\leq 6 \text{ mW/cm}^2$ . Because of graphical reasons the results of only three of the five irradiation frequencies used are presented in this figure.

sensitivity of the chromosomes to i) an overall temperature increase and ii) to small temperature gradients was examined. For i) the sham-exposed sample was warmed up by  $3^\circ\text{C}$  over the temperature of the control which corresponds to about ten times the microwave-induced temperature rise. In the case of ii) the microwave induced temperature gradient was simulated by use of incoherent far-infrared-radiation of a wavelength  $20 \mu\text{m} \leq \lambda \leq 70 \mu\text{m}$ . For that a globar was used as thermal source in combination with a KRS-5-lens, a germanium lens- and a PTFE-filter (thickness: 1 mm).

In both cases no significant difference between samples in the left and right chamber was found, thus confirming the nonthermal nature of the millimeter-wave irradiation effect (Table I). It should be noted that a localized heating of the sample above the temperature of the surrounding Cannon's medium is not possible due to heat conduction in the medium [15].

In view of the fact that the puffing phenomenon is not yet fully understood on a molecular basis, the explanation of the millimeter-wave effect must be speculative. Recently Edwards *et al.* [16a, 16b] found for supercoiled DNA of definite length a resonant absorption at microwave frequencies. This absorption is attributed to standing longitudinal acoustic waves along supercoiled DNA with an odd

Table I. Regression probability after sham-, "IR" and millimeter-wave irradiation:  $n$  describes the number of gland pairs used in one type of experiment. The number in brackets indicates the number of chromosomes used for that experimental series.  $r$  is the mean of the percentage regression probability for the glands placed in the left chamber; resp. right chamber. The number in brackets indicates the standard error of the mean.  $P$  is the probability in percent that the samples in the left chamber belong to the same distribution as the samples in the right chamber according to the U-Test of Mann-Whitney [14].

Type	$n$	$r$ [%] Left chamber	$r$ [%] Right chamber	$P$ [%]
sham-exposure	12 (908)	0.2 ( $\pm 0.2$ )	0.0 ( $\pm 0.0$ )	36.3
sham-exposure with additional heating of $3^\circ\text{C}$	12 (891)	0.7 ( $\pm 0.6$ )	0.0 ( $\pm 0.0$ )	24.5
infrared irradiated sample ( $20 \leq \lambda \leq 70 \mu\text{m}$ )	10 (724)	0.9 ( $\pm 0.5$ )	0.5 ( $\pm 0.3$ )	32.6
$41.2 \pm 0.0005 \text{ GHz}$	14 (912)	6.4 ( $\pm 1.3$ )	0.4 ( $\pm 0.4$ )	0.1
$45.2 \pm 0.0005 \text{ GHz}$	10 (759)	7.2 ( $\pm 0.8$ )	0.4 ( $\pm 0.3$ )	0.007
$67.2 \pm 0.0005 \text{ GHz}$	13 (707)	6.9 ( $\pm 1.7$ )	0.8 ( $\pm 0.8$ )	0.2
$68.2 \pm 0.0005 \text{ GHz}$	17 (931)	6.0 ( $\pm 1.2$ )	0.7 ( $\pm 0.3$ )	0.1
$80.2 \pm 0.0005 \text{ GHz}$	14 (947)	6.4 ( $\pm 1.2$ )	0.0 ( $\pm 0.0$ )	0.007

number of half wavelengths. It was predicted by Kohli *et al.* [17]. Transforming the DNA by treatment with the enzyme topoisomerase I into relaxed DNA strongly increases the resonant absorption [18]. The decondensed DNA in the Balbianirings of giant chromosomes is relaxed DNA of one definite length. One might speculate that microwave-absorption via longitudinal acoustic modes could specifically lead to an interaction of the electromagnetic field with the decondensed DNA in the Balbianirings and thus destabilize them. The DNA in the Balbianiring BR 2 has a length of about  $5\text{--}6 \mu\text{m}$  according to Sass [19]. Edwards *et al.* [16] found for linear DNA of 1792 base pairs (corresponding to a length of about 600 nm) dissolved in storage buffer a resonance near 4 GHz (resonance half-width: about 1 GHz) which was assigned to the lowest mode according to

$$(n + \frac{1}{2}) \cdot \lambda = l; \nu_s = \lambda \cdot v$$

where  $l$  is the length of the molecule,  $\nu$  the frequency of the resonance,  $n$  the  $n^{\text{th}}$  mode, the fundamental being described by  $n$  equal to zero and  $v_s$  the sound velocity. The latter was measured by Hakim *et al.* [20] as 1.69 km/s, which compares well with the value of 1.67 km/s as measured by Edwards *et al.* [16a/b]. Hence for DNA of a length of about 5  $\mu\text{m}$ , the absorption resonances are so dense that a broadband absorption at millimeter-wave frequencies would result, leading to a broadband radiation effect as observed.

It might be surprising that only the BR 2 and not the other Balbianirings were affected by the millimeter-wave irradiation. But the BR 2 of *Acricotopus lucidus* shows some special features compared to

other Balbianirings: The sensitivity to gibberellins [10, 11], its marked size contrasting to a relatively low transcription activity [21] and its morphological separation in two regions originating from RNP-grana of different size [22].

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