STANDARD OPERATING PROCEDURE @ MMDN - UMR 1198

Protocol	**Drosophila exposure to 26GHz RF-EMF**
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1. PURPOSE

The procedure describes the materials and the protocols for maintenance and handling of Canton S *Drosophila melanogaster* within controlled conditions for a chronic exposure to 26GHz RF-EMF.

2. BACKGROUND

Wireless communications and technologies are swiftly and widely expanding around us. They now perfused our lives through remote controlled or mobile devices, leading to increasing chronical exposure to radiofrequency electromagnetic fields (RF-EMF). Insects are expected to be possibly vulnerable to RF-EMF in the FR2 range due to their millimetric size. Several studies did already investigated effects of RF-EMF at lower frequencies on insects, either in controlled or uncontrolled conditions. This study aims to evaluate high frequency RF-EMF effects of the 5th generation (G) in controlled condition on a well-establish insect model.

Canton-Special (CS) is one of the most-used wild-type strains in *Drosophila melanogaster* genetics studies. The CS stock was established by C. B. Bridges and chosen because of its low mutation rate. The strain has been used as a control in neurogenetics and behavioural studies ever since.

3. PROCEDURES

31. EQUIPEMENT

Field at Works GmbH designed and manufactured 2 identical reverberation chambers. One chamber is fed by a randomized 5G signal at 26 GHz with a field strength up to 61 V/m (10W/m). The second chamber serves as a sham control.

Both chambers are remotely monitored for field strength, temperature at different locations, humidity and operational parameters that could affect the experiment. All monitoring data are continuously transferred to a remote server. Chambers are mounted inside an incubator at 24.5±0.5°C, constantly vented (Figure 1).

Experimenters were kept blinded from which chamber the RF-EMF exposure is taking place.

Figure 1: Reverberation chambers mounted inside the incubator, the vials with the drosophila are inserted into the metallic sample holder cups.

32. ANIMAL MODEL, REARING & RF-EMF EXPOSURE

All experiments were performed using *Drosophila melanogaster* Canton Special (CS) strain (generous gift from Dr Jean-Maurice Dura, IGH Montpellier, France). Rearing inside chambers was done in the dark at 24±0.5°C.

Standardised breeding method was used all along the study for each generation, every vial contained 20 females and 6 males to ensure proper fertilisation. Flies were left to lay eggs for 3 days, then the parents were removed.

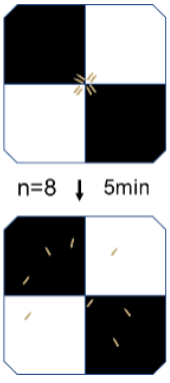
33. EXPOSITION

Flies were set to breed or to age in polypropylene vial containing 2.5 cm of standard fly media (BioCampus, CNRS, Montpellier), this ensured a proper reverberation of 26 GHz RF-EMF in the vial holder. Flies were maintained all along their life inside the reverberation chamber.

34. LARVAE L3 LOCOMOTOR ACTIVITY

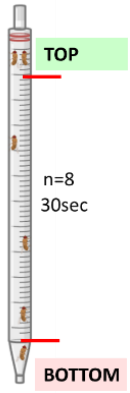
At appropriate generation (1st, 5th and 10th), Larvae L3 were collected and placed at the centre of a 14cm plastic dish filled with 2% agarose PBS 1X, larvae were left for 1 min to acclimate. Larvae were filmed for 2 min using ZebraBox system (Viewpoint, Lyon, France) and movies were analysed using the Videotracking Software (Viewpoint, Lyon, France). Total travelled distance, average speed and the motility index were quantified and analysed. The results were shown as mean ± SEM. The experiment was carried on 34 replicates at each generation, each replicate containing 3 larvae from RC1 and RC2, to reach n=100.

35. LARVAE L3 LIGHT-DARK PREFERENCE INDEX



For this experiment, 14cm plastic dishes containing 2% agarose PBS 1X, were placed on a 4 quadrants slide, 2 quadrants are opaque to light. Prior to experiment, larvae L3 from RC1 and RC2 were kept in the dark. Eight L3 instar larvae from RC1 and RC2 were placed at the centre of the dishes. Dishes were set on a homogenous light field. L3 instar larvae were left to crawl freely between dark and transparent quadrant for 5 min. After, larvae in the transparent or dark area were quantified and phototaxis index was measured as follow: [number of larvae in light half/(number of larvae in the dark half + number of larvae in the light half)]. Light preference index was shown as a mean ± SEM. The experiment carried 13 replicates to reach n=100 larvae per reverberation chamber.

36. NEGATIVE GEOTAXIS (CLIMBING PERFORMANCES)



At appropriate generations (1st, 5th and 10th), freshly hatched female flies were briefly anesthetised using CO2, then 25 females were isolated per vials. Five vials per generation and chamber were prepared and left to age inside reverberation chambers. Vials were renewed every 2 days. Climbing performances were determined using the reflex of flies to walk against gravity (negative geotaxis) at 10 and 21 days old. Flies were anesthetised using CO2. Eight flies were placed in a clean plastic column (1.3 cm diameter). Flies were left to recover from anaesthesia for 20 min inside an incubator set at 24°C in the dark. After being gently tapped, flies that remained at the bottom or crossed the top mark at 22 cm were counted after 30 sec. The test was repeated three times for each batch of flies. The data are the mean of at least five trials and are expressed as percent ± SEM of the total number of flies.

37. PSEUDOPUPIL ANALYSIS

At appropriate generation (2nd, 4th, 6th and 8th), freshly hatched adult flies were collected, and anesthetised with CO2. Flies were then kept on ice, and beheaded. Heads were placed on vaseline coated slide with an appropriate angle. Pseudopupils were imaged using a brightfield microscope. The number of photoreceptors per ommatidia was determined from at least 20 ommatidia per fly. The number of photoreceptors per ommatidia were quantified using FIJI cell-counter plugin. Results were shown as mean ± SEM from 12 flies per RC.

38. OMICS ANALYSIS

At appropriate generations (1st, 5th and 10th) flies were collected at day 0 (freshly hatched) and 35 days old. Aging flies were maintained and vial were renewed every 2 days. At collection day, flies were anesthetised on CO2 and snap frozen in liquid nitrogen, then stored at -80°C prior to further analysis.

- For metabolomics

Samples containing 10 flies per cryotubes were transferred to MAMMA-PLATON platform (CNRS, Montpellier) on dry-ice. Five samples per conditions were provided. Samples were processed following their in-house protocols.

- For transcriptomics

RNAs were extracted using the Monarch total RNA miniprep Kit (#T2020S, New England, Biolab). Sample quality was tested using Agilent micro-chip. Five samples of 2µg each were transferred on in dry-ice to MGX-Platform (CNRS, Montpellier). RNAseq was performed according to their in-house protocols.