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REPORT **報告**
UNDER THE AGREEMENT ON SCIENTIFIC COOPERATION WITH AIRES HUMAN GENOME RESEARCH FOUNDATION Subject: Study of high-frequency electromagnetic radiation impact and Aires resonators influence on behavior, genetic and epigenetic processes in cells of central and peripheral organs (models organisms: rat (Rattus norvegicus) and honeybee (Apis mellifera L.)

根據與 AIRES HUMAN GENOME RESEARCH FOUNDATION 的科學合作協議 主題：高頻電磁輻射影響及 Aires 共振器對中樞與周邊器官細胞之行為、遺傳與表觀遺傳過程的研究（模式生物：鼠類（Rattus norvegicus）與蜜蜂（Apis mellifera L.））

FIFTH STAGE: Study of the effect of «Aires Defender Pro» resonators on the expression of the stress-reactive $hsp70$ gene in the brain of a honeybee.

第五階段：研究 «Aires Defender Pro» 共振器對蜜蜂腦部壓力反應性 $hsp70$ 基因表現之影響。

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REPORT **報告**

UNDER AGREEMENT ON COOPERATION IN SCIENCE BETWEEN FEDERAL STATE BUDGET-FUNDED INSTITUTION OF SCIENCE PAVLOV INSTITUTE OF PHYSIOLOGY OF THE RUSSIAN ACADEMY OF SCIENCE AND AIRES HUMAN GENOME RESEARCH FOUNDATION

基於俄羅斯科學院巴甫洛夫生理研究所（聯邦國家預算資助科學機構）與 AIRES 人類基因組研究基金會之間科學合作協議

Subject: **主題：**

Study of High-Frequency Electromagnetic Radiation Impact and Aires Resonators Influence on Behavior, Genetic and Epigenetic Processes in Cells of Central and Peripheral Organs (Rattus norvegicus and Apis mellifera L.Honey Bee Models)

高頻電磁輻射影響與 Aires 共振器對中樞與周邊器官細胞行為、基因與表觀遺傳過程之研究（以褐家鼠 Rattus norvegicus 與蜜蜂 Apis mellifera L. 為模式）

STAGE FIVE (December - June, 2019): Study of Aires Resonators Influence on StressResponsive hsp70 Gene Expression in the Honey Bee Brain.

第五階段（十二月–六月，2019 年）：研究 Aires 共振器對蜜蜂腦中應激相關 hsp70 基因表現的影響。

INTRODUCTION 引言

Over millions of years natural electromagnetic fields (geomagnetic field, solar radiation, atmospheric electricity) have been a constant ecological factor, influencing condition of living organisms and ecosystems. Evolving organisms adapted to influence thereof. Today, as a result of progress in science and technology, there is a great number of electric devices, helping a man in various areas of life. Most electric devices around us are sources of electromagnetic radiation of different frequency and power. High level of electromagnetic pollution of the environment can be harmful to living organisms. Degree of EMR impact on living beings depends on field power and strength, oscillations frequency, exposure duration and mode of generation thereof (pulse and continuous fields) (Kudryashov et al., 2008).

數百萬年來，自然電磁場（地磁場、太陽輻射、大氣電力）一直是恆定的生態因子，影響著生物體與生態系的狀況。演化過程中，生物體逐漸適應這些影響。如今，隨著科學與技術的進步，大量電器設備在各個生活領域協助人類。我們周遭的多數電器都是不同頻率與功率的電磁輻射來源。環境中高強度的電磁污染可能對生物造成危害。電磁輻射對生物的影響程度取決於場的功率與強度、振盪頻率、曝露持續時間以及產生方式（脈衝場與連續場）（Kudryashov et al., 2008）。

Insects (butterflies, ants, cockroaches, flies) are deemed to be the best experimental animals to study EMR impact as they are highly sensitive to magnetic and electric fields (Kumar et al., 2011).

昆蟲（蝴蝶、螞蟻、蟑螂、蒼蠅）被認為是研究電磁輻射（EMR）影響的最佳實驗動物，因為它們對磁場和電場高度敏感（Kumar et al., 2011）。

It has already been proved that impact of high-frequency radiation decreases queen bee's fertility and leads to reduction of honey and bee-bread amount in a family (Kumar et al., 2011). Unconditional-reflex food excitability and short-time memory of a honey bee deteriorates (Lopatina et al., 2019). Molecular and cellular mechanisms of this phenomenon are

unknown and need careful study. Apparently, change of the natural electromagnetic background is a stressor for bees.

已有研究證實，高頻輻射會降低蜂王的生育能力，並導致蜂群中的蜂蜜與花粉餅量減少（Kumar et al., 2011）。蜜蜂的無條件反射式食物興奮性與短期記憶也會退化（Lopatina et al., 2019）。此現象的分子與細胞機制尚不清楚，需進一步深入研究。顯然，自然電磁背景的改變對蜜蜂而言是一種壓力源。

Heat shock proteins (HSP) are universal stress reaction sensors. Heat shock proteins are one of the most conservative and phylogenetically ancient proteins: homology degree of

熱休克蛋白（HSP）是普遍存在的壓力反應感測器。熱休克蛋白是最保守且在系統發生上最古老的蛋白質之一：同源程度為

eucaryotes and procaryotes HSP makes more than 50%, and some domains thereof are totally identical; structural similarity of human and mice HSP is up to 95%. A certain amount of heat shock proteins (HSP) is continuously synthesized in any nuclear cells, in numerous intracellular structures (in the nucleus, cytoplasm, endoplasmic reticulum, chloroplasts and mitochondria) of all multi-cellular organisms, regardless of exposure to stress factors. Heat shock proteins are molecular chaperones, participating in protein folding (tertiary structure formation), HSP prevent nonspecific proteins aggregation and protect them from premature proteolysis. HSP protect the cell from impact of mutant or misfolded proteins, from death of cells caused by stress. Increase of HSP intracellular synthesis is caused not only by heat shock, but also by any exposure to stress: external impact (UV, heavy metals, heat shock, amino acids), pathologic impact (viral, bacterial and parasitic infections, inflammation, malignant transformation, autoimmune response) or even physiological impact (growth factors, cell differentiation, hormonal stimulation, tissue growth) (Nikitin, 2008).

真核生物與原核生物的熱休克蛋白（HSP）含量超過 50% ，且其中某些區域完全相同；人類與小鼠 HSP 的結構相似度高達 95% 。在任何有核細胞中，無論是否受到應激因子影響，所有多細胞生物的眾多細胞內構造（包括細胞核、細胞質、內質網、葉綠體與線粒體）都持續合成一定量的熱休克蛋白。熱休克蛋白為分子伴侶，參與蛋白質摺疊（形成三度空間結構），HSP 可防止非專一性的蛋白質聚集並保護它們免於過早被蛋白酶分解。HSP 保護細胞免受突變或錯誤摺疊蛋白的衝擊，以及因應激所致的細胞死亡。細胞內 HSP 合成的增加不僅由熱休克引起，任何形式的應激都會誘發其上升：外在刺激（紫外線、重金屬、熱休克、胺基酸）、病理性刺激（病毒、細菌與寄生蟲感染、發炎、惡性轉化、自體免疫反應）甚至生理性刺激（生長因子、細胞分化、荷爾蒙刺激、組織生長）（Nikitin, 2008）。

HSP70 heat shock protein (which belongs to the family of proteins with molecular mass more than 70 kDa) is the most studied one. HSP70 acts as a chaperone in the cell, besides it participates in stress-related processes, such as aggregation, deaggregation, degeneration and restoration of three-dimensional folding (Nikitin, 2008).

HSP70 熱休克蛋白（屬於分子量超過 70 kDa 的蛋白質家族）是研究最深入的一類。HSP70 在細胞中擔任伴護蛋白的角色，並參與與壓力相關的過程，例如蛋白聚集、解聚、退化以及三維折疊的恢復（Nikitin, 2008）。

According to the data base NCBI GenBank (LOC408706 heat shock protein 70Cb ortholog), gene length of a honey bee makes 8361 bps. Length of mRNA: XM_623196.5-4605 bps, XM_006561162 - 4497 bps. Proteins length: XP_006561225.1 - 831 a.a., XP_623199.2 861 a.a.

根據資料庫 NCBI GenBank（LOC408706 heat shock protein 70Cb 同源基因），蜜蜂的基因長度為 8361 個鹼基對。mRNA 長度：XM_623196.5 為 4605 個鹼基，XM_006561162 為 4497 個鹼基。蛋白質長度：XP_006561225.1 為 831 個胺基酸，XP_623199.2 為 861 個胺基酸。

Purpose of this work is to study stress-responsive hsp70 gene expression in the honey bee Brain upon exposure to electromagnetic radiation, emitted by WiFi router and simultaneous exposure to WiFi router and Aires Defender Pro resonators.

本研究旨在探討在暴露於由 WiFi 路由器所發射的電磁輻射，以及同時暴露於 WiFi 路由器與 Aires Defender Pro 共振器時，蜂蜜蜂腦中對壓力反應的 hsp70 基因表現。

STUDY MATERIALS AND METHODS

研究材料與方法

The work was performed using 10-30 day Apis mellifera carnica worker honey bees. Bees were bred at the bee house of the Pavlov Institute of Physiology of the Russian Academy of Science. The bees meant for the experiment were kept in the observation queen-bee cell in the special premise at a room temperature and automatic lighting from 8 a.m. to 8 p.m. .

實驗使用年齡為 10 至 30 天的 Apis mellifera carnica 工蜂。蜜蜂在俄羅斯科學院巴甫洛夫生理研究所的蜂房繁殖。待用於實驗的蜜蜂被置於觀察用蜂王細胞中，放在特別房間內，室溫保存並於每天上午 8 時至下午 8 時提供自動照明。

5 groups of bees participated in the experiment ($10-16$ animal units per each group): intact group, control group (faraday's cage, isolation from external EMR), control group (6 Aires Defender Pro resonators in the center of each faraday's cage face), experimental group (WiFi

5 組蜜蜂參與了實驗（每組 $10-16$ 動物單位）：完整組、控制組（法拉第籠，隔離外來電磁輻射）、控制組（每個法拉第籠面中心放置 6 個 Aires Defender Pro 共振器）、實驗組（WiFi

router operating in the faraday's cage in the 24 h mode +6 Aires Defender Pro resonators resonators).

在法拉第籠內以 24 小時模式運作的路由器 +6 個 Aires Defender Pro 共振器。

The following algorithm was used to study impact of electromagnetic waves on hsp70 gene expression: exposure to EMR router (2 groups of bees), control without exposure (3 groups of bees), extraction of RNA from brains of bees of all groups, RT-PCR with electrophoretic detection (Fig. 1, 2).

以下流程用來研究電磁波對 hsp70 基因表現的影響：暴露於無線路由器電磁輻射（2 組蜜蜂）、未暴露之對照組（3 組蜜蜂）、從所有組別蜜蜂的大腦萃取 RNA、進行 RT-PCR 並以電泳檢測（圖 1、2）。

Figure 1: Groups of bees under study

圖 1：受試蜜蜂群組

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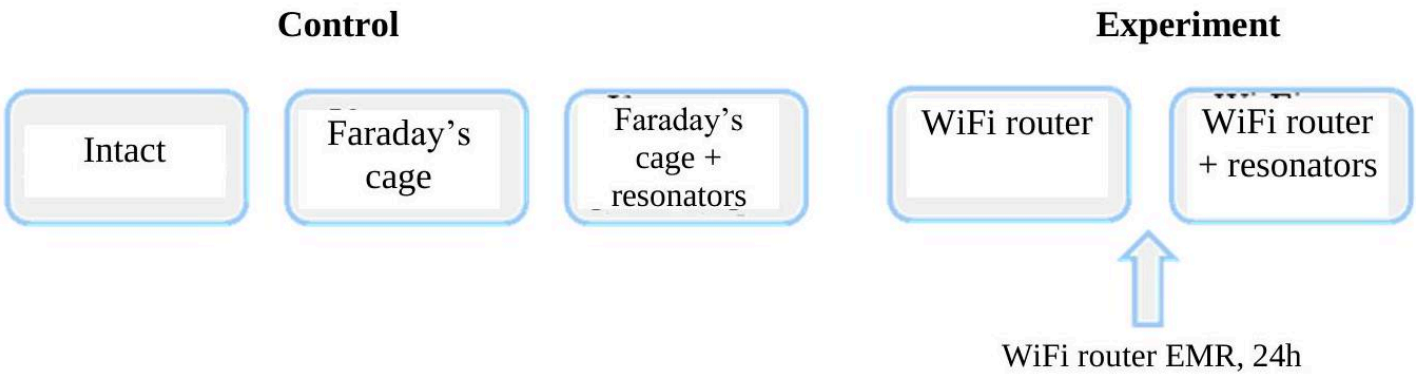

Figure 2: Evaluation of hsp70 gene expression

圖 2：hsp70 基因表現評估

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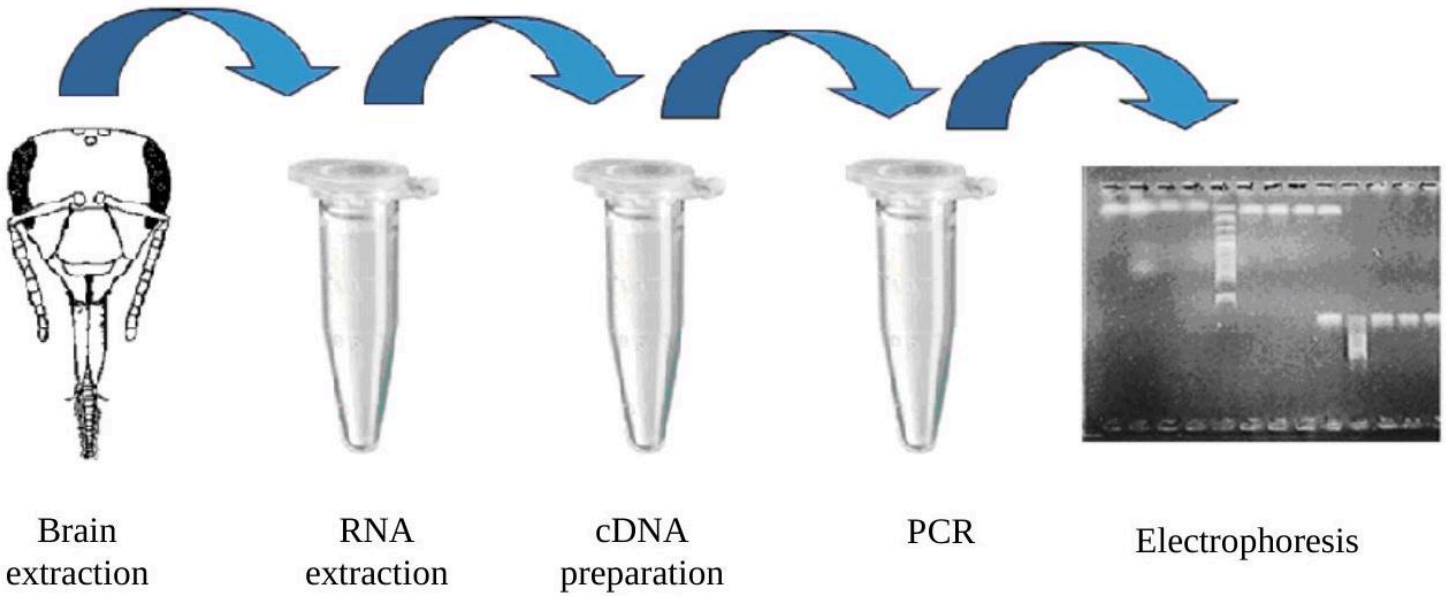

Fig. 1. Design of the experiment

圖 1。實驗設計

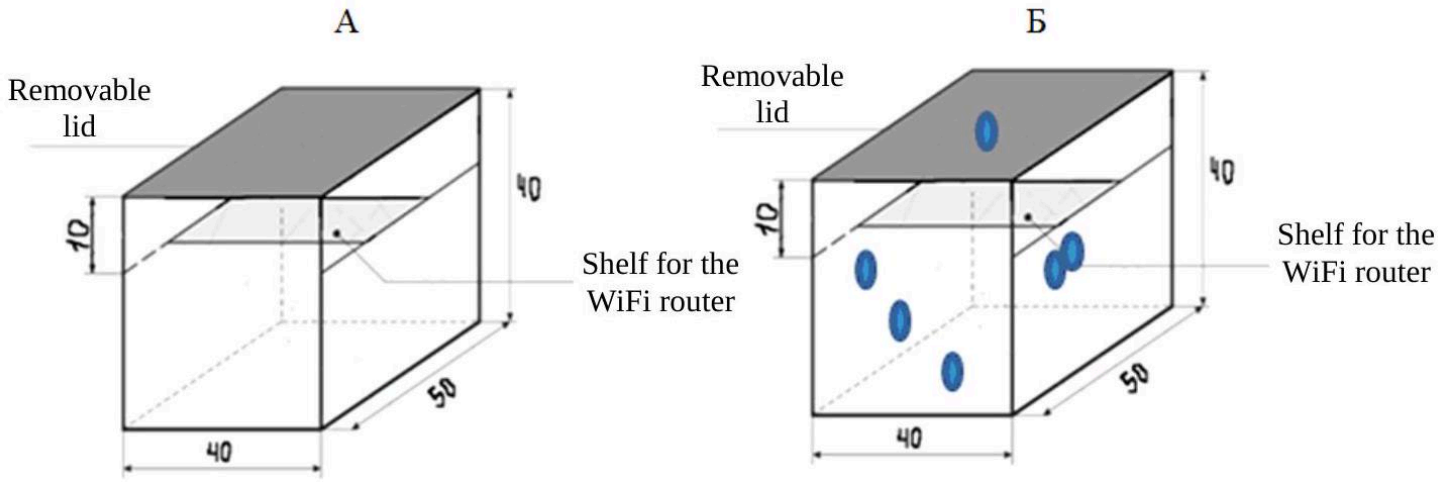

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Figure 3: Fig. 2. А - Faraday's cage, Б - Faraday's cage + Aires Defender Pro resonators.

圖 3：圖 2。А - 法拉第籠，Б - 法拉第籠 + Aires Defender Pro 共振器。

Extraction of total RNA from the brain of the honey bee. The bees were taken from the hive and immediately placed into the freezer for 10 minutes. Afterwards, the head capsule

從蜜蜂腦部萃取總 RNA。蜜蜂從蜂巢取出後立即置於冰箱冷凍 10 分鐘。接著，頭殼蓋體...

was opened up and the brain was extracted. The brain was homogenized with a pestle in 1.5 mcl centrifugal tube with 200 mcl homogenization buffer (Evrogen). The homogenate was incubated at a room temperature during 10 minutes. 300 mcl of chloroforme was added for deproteinization, samples were thoroughly vortexed. The samples were incubated at a room temperature during 5 minutes. Then they were centrifugated at $13,000\text{rpm}$ during 3 minutes. The supernatant with nucleic acids was transferred to a clean test tube. 500 mcl of isopropyl alcohol was added, the sample was mixed. 100 mcl of 3 M Sodium acetate was added, the sample was carefully mixed. The samples were placed into the refrigerator at -20°C for 1 hour for nucleic acids to precipitate. Then the samples were centrifugated at $13,000\text{rpm}$ during 3 minutes. The supernatant was removed. 500 mcl of 96% ethyl alcohol was added, the samples were mixed. Then they were centrifugated at $13,000\text{rpm}$ during 3 minutes. The supernatant was withdrawn. 500 mcl of 70% ethyl alcohol was added, the samples were mixed. Then they were centrifugated at $13,000\text{rpm}$ during 3 minutes. The supernatant was withdrawn. 500 mcl of 70% ethyl alcohol was added, the samples were mixed. Then they were centrifugated at $13,000\text{rpm}$ during 3 minutes. The supernatant was withdrawn. The wash-out was repeated (steps 16-18). The sediment was diluted in TE buffer, pH 8.0 (1 mM EDTA, 10 mM tris hydrochloride, pH 8.0) at a room temperature during 10 minutes. The samples of the total RNA,

prepared in such a way, were stored at −20°C.

解剖開顱並取出腦組織。腦組織放入 1.5 mcl 離心管中，加入 200 mcl 研磨緩衝液（Evrogen），用乳杵研磨均質。均質液於室溫孵育 10 分鐘。加入 300 mcl 氯仿以去蛋白，樣品徹底漩渦混合。樣品於室溫孵育 5 分鐘。接著以 13,000rpm 離心 3 分鐘。含核酸的上清液轉移至乾淨的試管。加入 500 mcl 異丙醇，混合樣品。加入 100 mcl 3 M 乙酸鈉，小心混合樣品。將樣品置於 −20°C 冰箱中冷藏 1 小時，使核酸沉澱。然後以 13,000rpm 離心 3 分鐘。移除上清液。加入 500 mcl 96% 乙醇，混合樣品。接著以 13,000rpm 離心 3 分鐘。抽出上清液。加入 500 mcl 70% 乙醇，混合樣品。接著以 13,000rpm 離心 3 分鐘。抽出上清液。加入 500 mcl 70% 乙醇，混合樣品。 然後以 13,000rpm 離心 3 分鐘。上清液被移除。洗滌步驟重複（步驟 16-18）。沉澱物在室溫下以 TE 緩衝液（pH 8.0，1 mM EDTA，10 mM 三羥甲基氨基甲烷鹽酸鹽，pH 8.0）稀釋，靜置 10 分鐘。如此製備的總 RNA 樣本儲存在 −20°C 。

Reverse Transcription. The reaction of reverse transcription was performed using the obtained samples and the reverse transcription set (Evrogen) with a random primer (Evrogen) according to the manufacturer's recommendations (2 hours at 38°C). The obtained cDNA was stored at −20°C.

逆轉錄。逆轉錄反應使用所獲樣本及逆轉錄試劑組（Evrogen）與隨機引子（Evrogen）依製造商建議進行（在 38°C 進行 2 小時）。所得的 cDNA 儲存在 −20°C 。

Polymerase Chain Reaction. cDNA, obtained upon reverse transcription was used as a matrix. PCR was performed according to the manufacturer's recommendations (Evrogen), using Veriti 96-Well Thermal Cycler (Applied Biosystems). Primers annealing temperature was 61°C. Cycles number - 40. Primers (10pmol/mcl, Evrogen): they were selected by T.G. Zachepilo in the GenBank by means of PrimerBLAST online package.

聚合酶鏈反應。逆轉錄所得到的 cDNA 用作模板。PCR 依製造商（Evrogen）建議進行，使用 Veriti 96-Well Thermal Cycler（Applied Biosystems）。引子退火溫度為 61°C 。循環次數為 40 次。引子（ 10pmol/mcl ，Evrogen）：由 T.G. Zachepilo 使用 PrimerBLAST 線上工具在 GenBank 中選定。

Table 1: Table 1. Primers

表 1：表 1. 引子

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Direct/reverse **正向/反向**	Sequence **序列**	Product **產物**	Primer **引子 (Primer)**
Direct **正向**	Apis mellifera heat shock protein 70Cb ortholog (LOC408706), XM_623196.5 **Apis mellifera heat shock protein 70Cb 同源基因 (LOC408706), XM_623196.5**	92bps **92bp**	AAGCACAAGCAAATGAACCACCG
Reverse **反向**			CCTCGCACCTCTTCCACCAT
Direct **正向**	Apis mellifera ribosomal protein L32 (RpL32), NM_001011587.1 **Apis mellifera 核糖體蛋白 L32 (RpL32), NM_001011587.1**	109bps	TGTGCTGAAATTGCTCATGGT
Reverse **反向**			AGAACGTAACCTTGCACTGG

Electrophoresis in Agarose Gel. PCR-products were mixed with the loading buffer (Evrogen, 1:1). DNA fragments were separated in 10x15cm 1.5% agarose gel (with admixture of ethidium bromide) with TAE buffer in the horizontal electrophoresis chamber (Helicon) at 150 V during 40 minutes. Do determine the size of the amplificated fragments, DNA-markers were

瓊脂糖凝膠電泳。將 PCR 產物與上樣緩衝液（Evrogen，1:1）混合。DNA 片段在含有溴化乙錠的 10x15 cm、1.5% 瓊脂糖凝膠中以 TAE 緩衝液進行水平電泳（Helicon 電泳槽），電壓為 150 V，時間為 40 分鐘。為了確定擴增片段的大小，於凝膠上加載了 DNA 尺標：100 bps（Evrogen）。

applied on the gel: 100bps (Evrogen). Electrophoresis results were detected in the transmitted ultraviolet, using the transilluminator (Vilber Lourmat). The gel was shot, using a digital camera. The results were saved on the computer in JPEG format.

電泳結果在透射紫外光下以轉燈（Vilber Lourmat）檢視。使用數位相機拍攝凝膠影像。結果以 JPEG 格式儲存於電腦中。

Data Processing. Photos of electrophoregrams were analyzed in ImageJ (NCBI). First, paths were identified in the image, then the area of stained bands was evaluated. Normalization was performed: ratio of area of $hsp70$ samples bands to the area of rp49 reference gene was determined.

資料處理。電泳影像照片在 ImageJ（NCBI）中分析。首先在影像中識別條帶路徑，接著評估染色條帶的面積。進行正規化處理：計算樣本 $hsp70$ 條帶面積與參考基因 rp49 條帶面積的比值。

Then pairwise comparison of normalized values was performed in all groups (total 10), using non-parametric Mann-Whitney test. Statistical analysis was performed in Statistica 10.

然後對所有組別（共 10 組）進行兩兩比較，採用非參數 Mann-Whitney 檢定。統計分析使用 Statistica 10 執行。

FINDINGS **研究結果**

Expression of hsp70 stress-responsive gene upon exposure of the honey bee to electromagnetic radiation was studied by the method of RT-PCR and electrophoretic detection.

利用 RT-PCR 與電泳檢測方法，研究了蜜蜂暴露於電磁輻射後 hsp70 壓力反應基因的表現情形。

As a result of performed experiments electrophoregrams were obtained (Fig. 3-5). Expression was compared by matching bands in electrophoregrams. Thick and intensely colored bands refer to strong expression, while thin and weakly colored ones refer to weak expression.

實驗所得電泳圖譜（圖 3–5）如上。透過比對電泳圖譜上的條帶來比較表現量。粗且顏色濃的條帶代表表現量高，而細且顏色淡的條帶則代表表現量低。

Hsp70 gene expression in the intact group of bees was found in 3 samples (each sample contains the material, consisting of 2 bees brains) of 4 , i.e. the animals differ by functional state of the CNS.

在未處理之控制組中，4 個樣本中有 3 個樣本（每個樣本包含由 2 隻蜜蜂大腦組成的材料）檢出 hsp70 基因表現，顯示動物在中樞神經系統功能狀態上存在差異。

In the resonator control group (6 resonators on all faces of the faraday's cage) hsp70 stress-responsive gene expression was more uniform than in the intact bees control group. It might be related to influence of the faraday's cage and resonators, equalizing functional state of the CNS.

在諧振器控制組（法拉第籠所有面上各放置 6 個諧振器）中，hsp70 應激反應基因的表現比完整蜜蜂對照組更為一致。這可能與法拉第籠與諧振器的影響有關，使中樞神經系統的功能狀態趨於均一化。

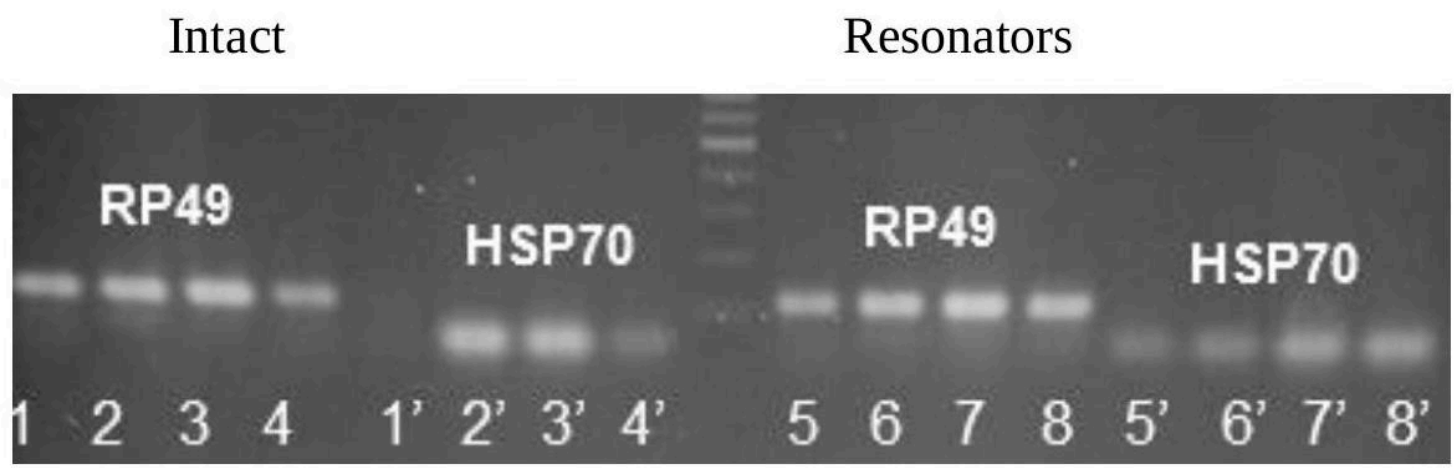

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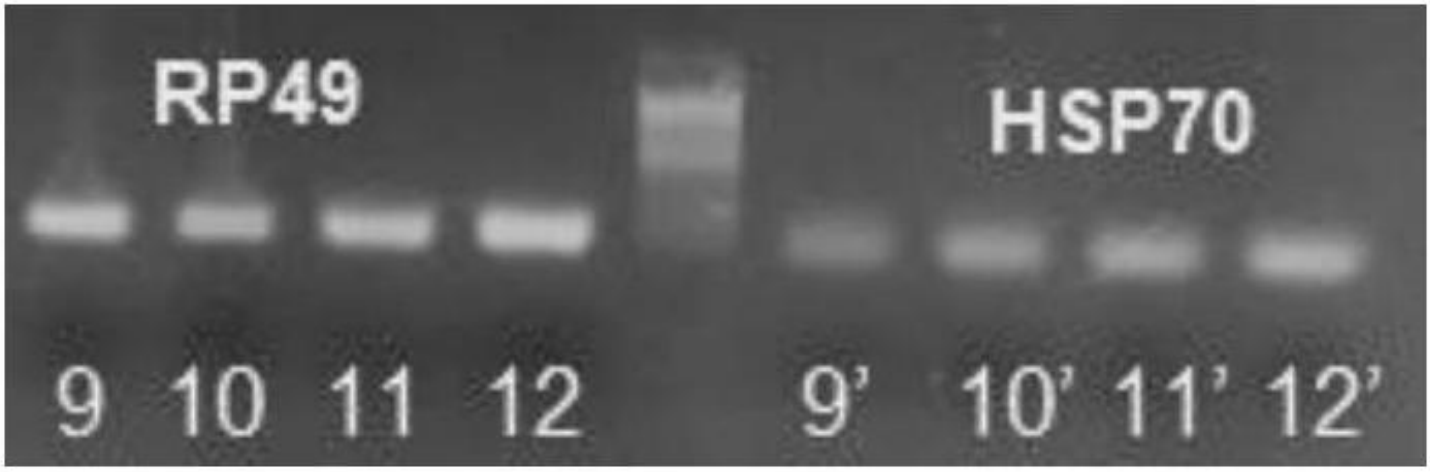
Figure 4: Fig. 3. Electrophoregram of PCR-products in the intact and resonator groups. Henceforth, figures are samples numbers, gene under study - hsp70, reference gene - rp49.

圖 4：圖 3。完整組與諧振器組的 PCR 產物電泳圖。此後，圖示為樣本編號，研究基因為 hsp70，參考基因為 rp49。

In the faraday's cage control group expression of hsp70 was similar to that of the resonator group. Thus, control groups of bees were the same, apart from the intact one.

在法拉第籠控制組中，hsp70 的表現與諧振器組相似。因此，蜜蜂的控制組除了完整組外，其餘皆相同。

Faraday's cage **法拉第籠**

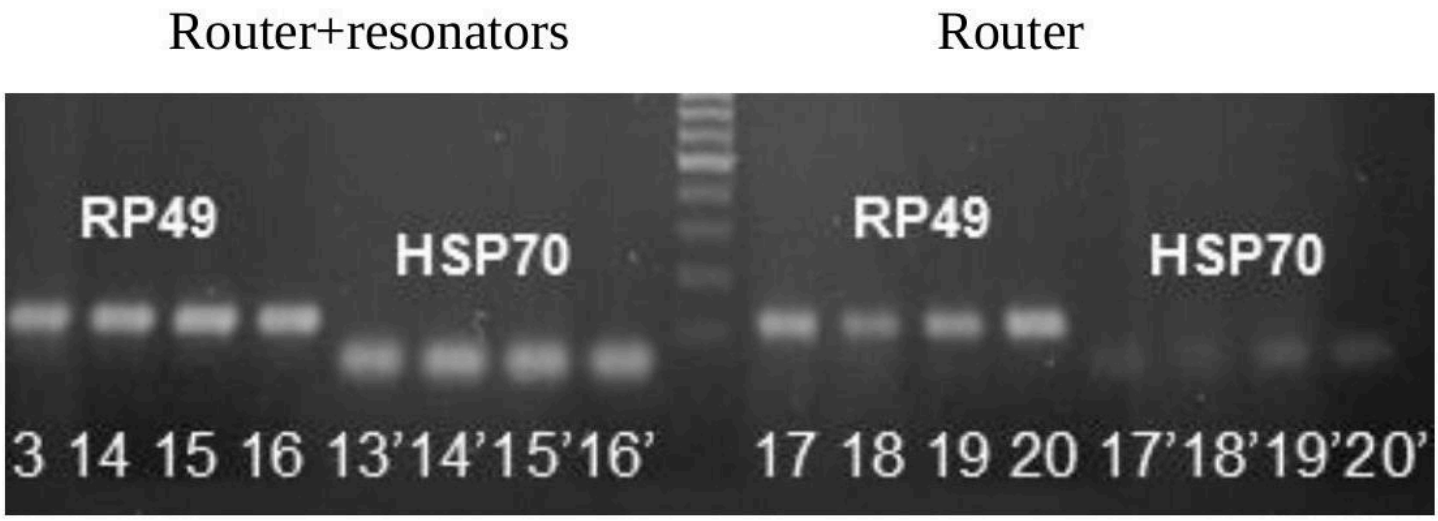


\captionsetup{labelformat=empty}
Figure 5: Fig. 4. Electrophoregram of PCR-products in the faraday's cage group

圖 5：圖 4。法拉第籠組的 PCR 產物電泳圖

In the router group hsp70 gene was expressed positively weaker, than in the faraday's cage and router+resonator groups. I.e. 24 h impact of EMR, emitted by the router, caused weakening of hsp70 expression.

在路由器組中，hsp70 基因表現為陽性但較弱，與法拉第籠組和路由器＋共振器組相比顯著較低。亦即，路由器所發出的電磁輻射經 24 小時影響，導致 hsp70 表現減弱。



\captionsetup{labelformat=empty}
Figure 6: Fig.5. Electrophoregram of PCR-products in the router+resonator and router groups

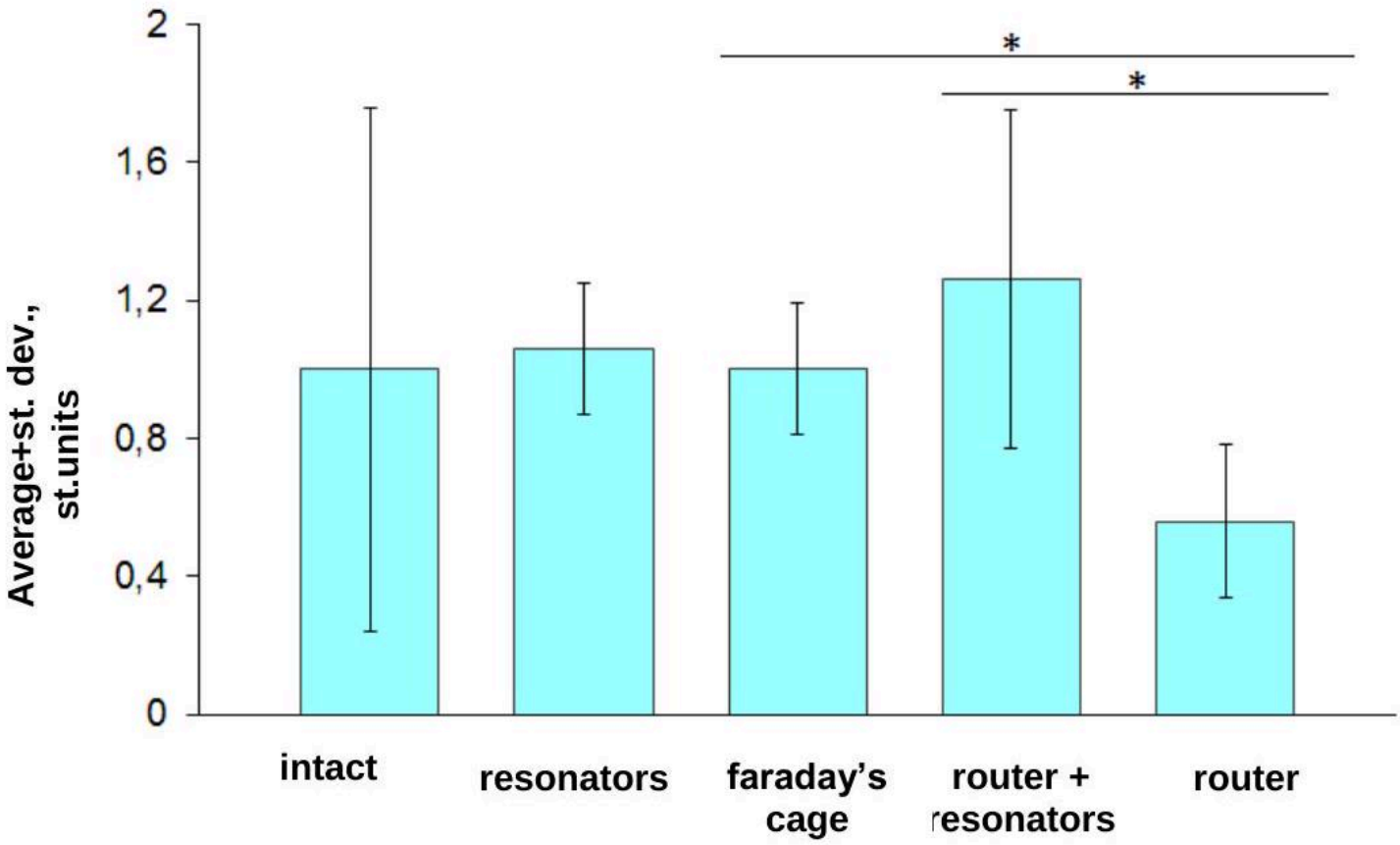
圖 6：圖 5。路由器＋共振器組與路由器組的 PCR 產物電泳圖

It is to be noted that any change of electromagnetic background, i.e. weakening of EMF in the faraday's cage, change of EMR parameters in the faraday's cage due to Aires Defender Pro resonators, EMR increase, caused by the router, result in change of hsp70 expression in comparison with the intact group.

值得注意的是，任何電磁背景的改變──例如法拉第籠內電磁場的減弱、因 Aires Defender Pro 共振器而改變的法拉第籠內電磁輻射參數、或由路由器引起的電磁輻射增加──都會導致與完整對照組相比 hsp70 表現的改變。

Results of data processing are summarized in Fig. 6.

資料處理結果總結如圖 6 所示。



\captionsetup{labelformat=empty}
Figure 7: Fig. 6. Expression of hsp70 gene in the bee's brain (normalized values _ standard deviation). * differences are significant, $p < 0,05$, Mann-Whitney test.

圖 7：圖 6。蜜蜂大腦中 hsp70 基因的表現（標準化數值 ± 標準差）。* 表示差異顯著，$p < 0,05$ ，Mann-Whitney 檢驗。

Thus it can be concluded as follows:

因此可作如下結論：

Level of hsp70 gene expression in the honey bee's brain is similar in control groups. Intact bees show greater variability of this parameter.

蜜蜂大腦中 hsp70 基因的表現水準在對照組間相近。完整未處理的蜜蜂在此指標上顯示較大的變異性。

After 24-h exposure to high-frequency electromagnetic radiation of a WiFi router, expression of hsp70 gene in the honey bee's brain weakens which can have negative impact on functioning of the bee's CNS.

在蜂群暴露於 WiFi 路由器的高頻電磁輻射 24 小時後，蜜蜂大腦中 hsp70 基因的表現減弱，這可能對蜜蜂中樞神經系統的功能產生負面影響。

Isolated 24 -h exposure to Aires Defender Pro resonators has no impact on expression of hsp70 gene in the honey bee's brain.

單獨讓蜜蜂暴露於 Aires Defender Pro 共振器 24 小時，對蜜蜂大腦中 hsp70 基因的表現沒有影響。

Upon simultaneous 24-h exposure to Aires Defender Pro resonators and WiFi router, expression of hsp70 gene in the honey bee's brain increases to the control level.

當蜜蜂同時暴露於 Aires Defender Pro 共振器與 WiFi 路由器 24 小時時，蜜蜂大腦中 hsp70 基因的表現回升至對照組水平。

DISCUSSION 討論

Heat shock proteins play an important part in the life of a cell and the organism as a whole. HSP participate in control of proteins quality, in protection of cells from aggregation of misfolded proteins or in forwarding of misfolded proteins to proteasomes for proteolysis thereof. Virtually all cell proteins at least temporarily interact with HSP70. Need in chaperones upon exposure to stress factors increases significantly. Interaction between the protein target and HSP70 result in stabilization of the former, then the protein folds correctly, or chaperons of a different type are recruited, which is followed by further restoration of the intact conformation (Nikitin, 2008). Thus decrease of transcriptional activity of hsp70 gene causes deficiency of HSP70 protein and increase of misfolded proteins and aggregates thereof. Accumulation of misfolded proteins and aggregates thereof in the neural tissue may result in derangement of learning processes and memory formation.

熱休克蛋白在細胞及整個生物體的生命活動中扮演重要角色。HSP 參與蛋白質品質的管控、保護細胞免於錯摺蛋白聚集，或將錯摺蛋白送往蛋白體（proteasome）進行蛋白質分解。幾乎所有細胞蛋白至少暫時都會與 HSP70 交互作用。暴露於壓力因子時對伴護蛋白（chaperone）的需求顯著增加。目標蛋白與 HSP70 的相互作用會先使前者穩定，接著蛋白得以正確摺疊，或是動員其他類型的伴護蛋白，進而恢復出完整的構象（Nikitin, 2008）。因此 hsp70 基因轉錄活性的降低會導致 HSP70 蛋白不足，並增加錯摺蛋白及其聚集體的數量。神經組織中錯摺蛋白及其聚集體的累積，可能導致學習過程和記憶形成的障礙。

Despite the fact that development of stress reactions is generally related to increase of HSP70 level, there are also evidences that such conditions as hyperthermia, ageing or disease may decrease reaction of heat shock proteins in the brain (Pardue et al., 2007). This demonstrates that increase of heat shock proteins synthesis can be necessary in some cell reactions, however, not in all of them (Agustiño et al., 2012). The literature also describes decrease of HSP70 in case of cerebral ischaemia (Yang et al., 2005). These data show that increased expression of HSP70 is not critical upon early adaptation. However, regulation at later stages, including increase of heat shock proteins number, suggests that stress proteins are of importance in facilitation of longterm tolerance.

儘管壓力反應的發展通常與 HSP70 水平上升有關，但也有證據顯示一些情況如高熱、老化或疾病可能會降低大腦熱休克蛋白的反應（Pardue et al., 2007）。這說明熱休克蛋白合成的增加在某些細胞反應中可能是必要的，然而並非在所有反應中都如此（Agustiño et al., 2012）。文獻也描述在腦缺血情形下 HSP70 會減少（Yang et al., 2005）。這些資料顯示 HSP70 的表現增加並非早期適應的關鍵。然而，在後期階段的調控，包括熱休克蛋白數量的增加，則暗示壓力蛋白在促進長期耐受性方面具有重要性。

Decrease in number of heat shock proteins is, apparently, indicative of existence of nonthermal physical stimuli, acting through unidentified mechanisms via low-intensity electric fields

熱休克蛋白數量的減少，顯然暗示存在透過未明機制、以低強度電場作用的非熱物理刺激

without direct connection between power and effect size. Since the animals were exposed to nonionizing radiation in their entirety, their organism could react to the stress in multiple ways (Agustiño et al., 2012).

而非功率與效應大小之間的直接關聯。由於動物是整體暴露於非游離輻射之下，其機體可能以多種方式對壓力作出反應（Agustiño et al., 2012）。

A number of works demonstrate negative impact of EMR on honey bees (Harst et al., 2006). Under impact of EMR, their locomotor activity decreased, their ability to orient in space was impaired, it took them more time to get to the hive, their ability to return to their family degraded abruptly, they built less and became more aggressive. It is shown that bees perceive EMR as danger signal (Favre, 2011). When exposed to EMR, egg-laying capacity of the queen bee decreased, drone brood was observed (Halabi et al., 2013), amount of honey and bee-bread in hives decreased abruptly (Kumar et al., 2011). Behavior deviations of a honey bee as a result of exposure to different-frequency EMR are also shown in works of Russian researchers (Yeskov, Bragin, 1986; Yeskov, Toboev, 2008; Lopatina et al., 2019).

許多研究顯示電磁輻射（EMR）對蜜蜂有負面影響（Harst et al., 2006）。在電磁輻射作用下，牠們的運動活動減少，定向能力受損，回巢所需時間增加，返回蜂群的能力急遽下降，築巢減少且變得更具攻擊性。研究顯示蜜蜂將電磁輻射視為危險訊號（Favre, 2011）。暴露於電磁輻射時，蜂王的產卵能力下降，出現無雄性的幼蟲（drone brood）（Halabi et al., 2013），蜂巢中的蜂蜜與蜂糧數量急遽減少（Kumar et al., 2011）。俄羅斯研究者的研究亦顯示蜜蜂在不同頻率電磁輻射暴露下的行為偏差（Yeskov, Bragin, 1986; Yeskov, Toboev, 2008; Lopatina et al., 2019）。

Similar ecologically significant changes in behavior and reproduction of other insects (locust, flies, ants) under EMR impact are demonstrated in the work of Cucurachi et al. (2013). It is shown that upon exposure to high-power EMR oxidative stress and change of genes expression are observed in Drosophila (Manta et al., 2017). CNS of insects is quite sensitive to EMR: it is shown that it causes decrease of ability to form conditioned food reflex to olfactic and visual stimuli in Myrmica sabuleti

ants (Cammaerts et al., 2011).

Cucurachi 等人（2013）的研究顯示，在電磁輻射（EMR）影響下，其他昆蟲（蝗蟲、蒼蠅、螞蟻）的行為與繁殖也出現了類似具有生態意義的顯著變化。另有研究顯示，暴露於高功率電磁輻射會在果蠅中觀察到氧化壓力增加與基因表現改變（Manta 等，2017）。昆蟲的中樞神經系統對電磁輻射相當敏感：研究指出，對 Myrmica sabuleti 螞蟻而言，電磁輻射會降低其對嗅覺與視覺刺激形成條件化食物反射的能力（Cammaerts 等，2011）。

Thus distortions of cognitive activity of honey bees upon long-term exposure to highfrequency electromagnetic radiation may be related to accumulation of misfolded neuronal proteins, caused by decrease of transcriptional activity of hsp70 gene and deficiency of HSP70 protein.

因此，蜜蜂在長期暴露於高頻電磁輻射後認知活動的扭曲，可能與神經元錯誤折疊蛋白質的累積有關，而這些錯誤折疊的累積是由於 hsp70 基因轉錄活性降低以及 HSP70 蛋白質缺乏所導致。

Results of the performed experiments expressly testify hsp70 expression normalization under influence of Aires Defender Pro resonators. The data, obtained during the series of experiments is partly in compliance with previously obtained data on food excitability and shortterm memory in bees upon simultaneous exposure to WiFi router and resonators. It is possible that hsp70 normalizing action of resonators has a deferred effect on bees' behavior.

进行的實驗結果明確證明在 Aires Defender Pro 共鳴器影響下 hsp70 表現恢復正常。這系列實驗所獲得的數據在某種程度上與先前關於蜂群在同時暴露於 WiFi 路由器與共鳴器時食物興奮性及短期記憶之既有資料相符。共鳴器對 hsp70 的正常化作用可能對蜜蜂行為具有延遲性影響。

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