Effects of extremely low frequency electromagnetic fields on turkeys

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ABSTRACT Several studies have examined the potential biological effects of electromagnetic fields (EMF) on birds; however, little attention has been paid to the extremely low frequency (ELF; 0–300 Hz; 0–50 μT) radiation found in an urbanized environment. For monitoring the effects of ELF EMF, we used a turkey (*Meleagris gallopavo*) model, because the nucleated erythrocytes of turkeys contain β-adrenoceptors, and norepinephrine- (NE-) activated β-adrenoceptors have an important role in physiological and behavioral processes. Our aims were the following: 1) to investigate the intracellular mechanisms; 2) to compare the intracellular mechanisms in the treated and control groups over time, considering inter-individual differences and intra-subject correlations; 3) and to study the reversible nature of the response. The turkeys in the treatment group were treated in vivo with ELF EMF (50 Hz; 10 μT) for 3 wk after a 1-wk-long adaptation period. The animals were not exposed to ELF EMF during the regeneration period (5 wk following the exposure). The NE-activated β-adrenoceptor function was detected by measuring the amount of 3′5′-cyclic-adenosine-monophosphate (cAMP), and the biochemical enzyme parameters were defined. Repeated measurements of cAMP levels were analyzed using marginal models and a piecewise linear mixed model to compare treatment and control groups over time. According to our results, NE-activated β-adrenoceptor function was decreased in the treated birds in a time-dependent manner, while there were no differences between toxicological parameters in the serum, compared to the normal ranges. The decreased NE-dependent β-adrenoceptor function could be compensated by the homeostatic complex during the 5-wk regeneration period. Extended experimental periods and more sophisticated analysis methods may help prevent harmful environmental effects on birds; furthermore, these findings could affect public health and the economy.

Key words: electromagnetic field exposure, β-adrenoceptor indication in turkeys, 3′5′-cyclic-adenosine-monophosphate, repeated measures analysis, piecewise linear mixed model

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INTRODUCTION

Spontaneous electromagnetic radiation (20 to 30 μT) has an effect on the natural selection of living organisms on Earth. Natural electromagnetic background radiation has been altered by technological inventions and innovations of modern civilization via extensive use of electric devices, which has resulted in increased electromagnetic fields (**EMF**).

There are several pieces of literature examining the potential biological effects of EMF (radiofrequency, microwaves); however, very few of them deal with the issue of whether and how extremely low frequency (**ELF**) radiation puts the welfare of humanity and/or the environment at any risk (Santini et al., 2009; Cifra et al., 2011). ELF EMFs are defined as frequencies between 0 to 300 Hz (Feychting et al., 2005; Funk et al., 2009).

Living organisms maintain equilibrium with their environment by adapting to changing external and internal circumstances. Behavior is a rapid biological answer to the environmental stimuli that can be detected easily, and it is investigated with relation of cause and effect of homeostatic physiological processes (Kavet and Banks, 1986; Palanza et al., 2008; Nagyeri et al., 2012).

In higher vertebrates, such as birds or mammals, the homeostatic physiological processes can be regulated at the cellular level by membrane function. Membrane potential has a role in the continuance of membrane function; therefore, examining membrane function in physiological processes and behavior is relevant. In this field

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the role of norepinephrine (**NE**) can be emphasized as the common mediator of behavior and physiological functions.

Monoaminergic trajectories play an important role in vegetative functions, such as regulation of the cardiovascular system and food uptake, thermostatic control, and circadian rhythm, and in the formation of behavior patterns seen in depression and anxiety (Ressler and Nemeroff, 2000). NE and its metabolites measured in urine, blood, and cerebrospinal fluid are elevated in panic disorders, post-traumatic stress disorders, special phobias, social anxiety, and generalized anxiety disorders, presenting a dysregulation of central and peripheral NE systems (Uhde et al., 1984; Ressler and Nemeroff, 1999).

Birds have been widely used for monitoring the environmental significance of exposure to nonionizing radiation (Balmori, 2009; Tomas et al., 2012). In the current study, a turkey (*Meleagris gallopavo*) model was designed to investigate the biological effects of physical parameters (frequency, field force) that are representative of commercially available electromagnetic devices. Turkeys were examined because they have nucleated erythrocytes, which can be used in studies of morphological changes at cellular level and are easy to separate. Nucleated erythrocytes of turkeys constitute a suitable model system for studying several ion transports and signal processes (Rudolph and Greengard, 1974). The erythrocyte membrane of turkeys contains β-adrenergic receptors, which are related to the 3′5′-cyclic-adenosine-monophosphate (**cAMP**) second messenger system (Gardner et al., 1975). β-adrenergic catecholamines (NE, epinephrine) can also enhance the intracellular cAMP level of the erythrocytes (Sutherland and Robison, 1966; James et al., 1994).

The effect of ELF EMF on the behavior of birds is detectable, but the results are inconsistent. The natural reversibility of cell functions is a remarkable fact that indicates the operation of a proper homeostatic system that can be worsened or improved by ELF EMF treatment. In the literature survey, the authors could not find any example of such a complex investigation for the detection of natural reversibility of cell functions after ELF EMF exposures.

Our research had multiple objectives:

1) We aimed to demonstrate the effect of in vivo ELF EMF treatment on in vitro levels of NE-activated β-adrenoceptor function of physiological processes as cellular mechanisms by taking parallel blood samples from treatment groups so as to verify result interpretation.
2) Our purpose was to estimate the means of cAMP levels in ELF EMF treated and untreated groups at distinct points in time to detect the differences at the level of NE-activated β-adrenoceptor mechanisms. Moreover, we aimed to characterize the rate of change in β-adrenoceptor function over time, while considering inter-individual differences and suitable intra-subject correlation structure.
3) In addition, our aim was to design the above mentioned experimental system to be able to point out the reversible nature of the response (β-adrenoceptor function) in a statistically verifiable way.

MATERIALS AND METHODS

Test Animals

Female adult turkeys (from state farm, weight 5,000 to 5,200 g) with veterinary certificates were used as the model system. The animal care and research protocols were in full accordance with the guidelines of the University of Szeged, Hungary.

Four animals served as control (one absolute control that was untreated; one positive control for which equipment was in standby mode; one negative control for which the machine was switched off; and one sham control that went through the protocol without receiving any EMF exposure). Forty animals were treated with intermittent ELF EMF. The animals were individually numbered.

Experimental Conditions

The turkeys were kept together during the experimental process except the treatment time. For the treatment, they were put separately in metal-free cages. At the beginning of the experiment, the animals were conditioned for 1 wk (adaptation period) to eliminate the possibility of stress effects caused by the new surroundings, animal caregiver, food, etc.

Extremely Low Frequency Electromagnetic Field Treatment

The treatment was performed using a special unit designed for the generation of pulsed ELF EMF (8 ms energy exposure – 2 ms energy free pause). During treatment, the cages were covered by a 200 cm × 80 cm "magnetic blanket" that was operated by the Hungarian electric regulation (U = 220 V, ν = 50 Hz). The turkeys were exposed to ν = 50 Hz (controlled by an electromagnetic equipment: ME3951A Low frequency (NF) analyzer, Gigahertz Solutions, Germany), B = 10 μT (checked by the PCE-EMF823 Electromagnetic Field Radiaton Tester, Tursdale Technicale Services Ltd, UK) intermittent ELF EMF treatment for 20 min every 8 h for the 3 wk of the experimental period.

ELF EMF Experimental Model

In Vivo Experiments (Figure 1/A)

Monitoring of Behavior The animals were regularly observed for physical activity (relaxation, play,

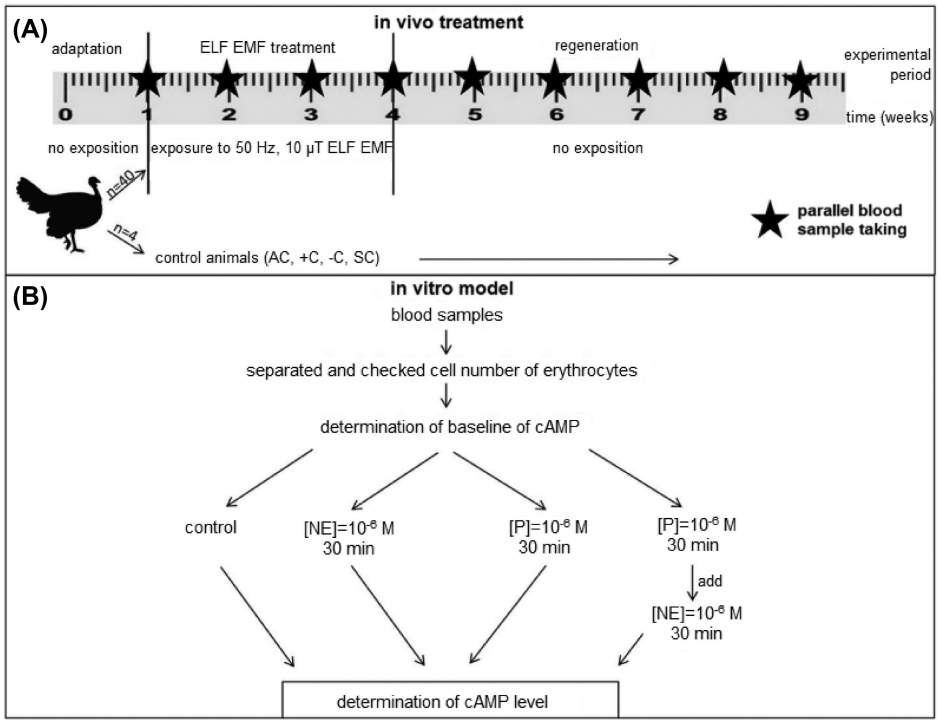

Figure 1. Structure of the ELF EMF experimental model. Notations: ELF, extremely low frequency; EMF, electromagnetic field; AC, absolute control; +C, positive control; −C, negative control; SC, sham control; cAMP, 3′5′-cyclic-adenosine-monophosphate; NE, norepinephrine; P, propranolol.

behavior, competition, aggression) and food and water consumption (Ficken and Ficken, 1966).

Blood Samples Heparinized blood samples were taken from the subclavian vein at the same time every wk during the examination. All measurements performed 4 to 6 technical parallels. Blood samples were separated by centrifugation for 5 min at 2,000 rpm. After removing the plasma, the red blood cells (**RBC**) were washed 3 times with 0.9% saline solution. At the end of the procedure, the erythrocytes were suspended in Ringer-solution buffered by 10 mM TRIS/HCl; pH 7.4 and the hematocrit value was 20%.

Toxicological Monitoring from Turkey Blood-Serum Enzyme Measurements During the experiment, the following biochemical enzyme parameters were measured from the blood: serum glutamic-oxaloacetic transaminase (**SGOT**) (Remaley and Wilding, 1989), serum glutamic-pyruvic transaminase (**SGPT**) (Matsuzawa et al., 1997), alkaline phosphatase (**AP**) and gamma-glutamyl transpeptidase (**γGT**) with Dialab methods (DIALAB production, Austria). Lactate dehydrogenase (**LDH**) activity was also measured (Remaley and Wilding, 1989).

In Vitro Experiments (Figure 1/B)

Incubation Protocol In the first in vitro experimental step, the baseline of intracellular cAMP was determined from all blood samples of the turkeys. For the study of the activation of β-adrenergic receptor, an agonist ([NE] = 10^{-6} M; t = 30 min) as well as an antagonist (propranolol (P): [P] = 10^{-6} M; t = 30 min) were used separately and combined. Combination means that the agonist was added at a concentration of 10^{-6} M to the erythrocytes following a 30 min preincubation with the antagonist at a concentration of 10^{-6} M. In all procedures, the intracellular cAMP level was measured after incubation.

Hemoglobin Determination The hemoglobin was transformed into cyanohemoglobin which was detected photometrically ($\lambda = 540$ nm) (O'Halloran et al., 1987).

Determination of cAMP Level In vitro samples were taken from RBC suspension (SUSP) of turkeys. These were denatured at 100°C for 3 min, and were kept at −20°C until further use. Before cAMP determination, the samples were defrosted and precipitated (at 0°C, 1600 rpm, t = 1 min). The cAMP content of samples was quantitated by competitive binding assay (Amersham cAMP Biotrak EIA system, GE Healthcare, UK).

Statistical Analysis

Means were calculated from technical replicates for each subject (experimental unit: turkey), and used for analyses of cAMP levels (as markers of β-adrenoceptor function). Descriptive statistics (mean, SD, n, minimum, median, maximum) were calculated to identify distribution of cAMP levels by treatment groups and time points (Supplemental Table 1 (Table S1)).

To verify the β-adrenoceptor function in the in vitro experimental model during the adaptation period (wk 1), cAMP levels were compared between groups of base, NE, P and P+NE, based on linear mixed model using random intercept for the subjects (Singer and

Willett, 2003; SAS, 2011). Mean cAMP levels of n = 40 animals (selected for ELF EMF treatment from wk 2) and SEs are presented.

To compare NE-activated β-adrenoceptor function of control animals (n = 4) to the average cAMP levels of turkeys (n = 40 and n = 44) at the adaptation period (wk 1), one-sample t-tests were applied. 95% CIs are presented with means and P-values ($\boldsymbol{P}$).

Effects of ELF EMF treatment on NE-activated β-adrenoceptor function were analyzed using a linear mixed model where cAMP levels of the treatment period (wk 2–4) as repeated measures of treated animals (n = 40) were compared to the adaptation period (wk 1).

To estimate means in ELF EMF treated and untreated groups over time in the whole experimental period (wk 1–9) considering between-subject differences and within-subject correlation, marginal model was applied using unstructured covariance structure (Zeger et al., 1988; Hamer and Simpson, 2000; Littell et al., 2000; Singer and Willett, 2003; SAS, 2011; Hayat and Hedlin, 2012). Differences of least squares means are calculated according to Sidak's adjustment.

To characterize the reversible nature of NE-activated β-adrenoceptor function after ELF EMF treatment by the rate of change in time, piecewise linear mixed model was used, which could describe the linear trajectories of cAMP levels in the treatment and in the regeneration periods (Naumova et al., 2001; Singer and Willett, 2003; Curran et al., 2010). An intraclass correlation coefficient was calculated to describe variation (Dickey, 2008).

In marginal and mixed models, the restricted maximum likelihood estimation method was used with unstructured covariance structure. Kenward-Roger's method was applied for adjusting df. Model residuals were checked for normality. All statistical analyses were performed using SAS (Version 9.3 SAS Institute Inc., Cary, NC, USA), with Type I error $\alpha = 0.05$.

RESULTS

In Vivo and In Vitro Combined Experimental Model

According to our observations, turkeys were more inactive depending on the duration of in vivo ELF EMF exposure. They did not show an interest in each other, scratched less, fluffed up their feathers, slept more, and huddled at the back of the enclosure. Despite the depression-related behavior, the ELF EMF had no effect on blood biochemical parameters (content of hemoglobin, SGOT, SGPT, AP, γGT, LDH). These results supported the assumption that the 10 μT exposure was subtoxic. Standard, combined experimental model was used to investigate the physiological background of behavior deviation. After the in vivo exposure, the function of β-adrenoceptors was detected in the in vitro functional model, which was performed.

To verify functionality of the in vitro model, the effect of the agonist (NE) was compared to the baseline, to the antagonist (P) and to P+NE of β-adrenoceptors (Figure 2). Multiple elevated levels of cAMP were detected following NE incubation under normal conditions. When P treatment was applied before NE administration, the NE-induced level of cAMP increase was blocked (mean ± SE cAMP (nmol/mL RBC SUSP): Base: 1.08 ± 0.02, NE: 16.60 ± 0.27, P: 1.13 ± 0.01, P+NE: 1.08 ± 0.02; $P < 0.001$ in comparison to NE vs. Base, NE vs. P and NE vs. P+NE). There were no differences ($P > 0.99$) between any other pairs of groups of Base, P or P+NE.

The intracellular levels of cAMP were determined from the blood samples for supporting the functionality of β-adrenoceptor in the examination protocol at the end of the adaptation period. Thus, all data could be used as self-control and also as untreated control at this time. Comparison of the mean cAMP level of the control group (absolute control; positive control; negative control; and sham control) to the ones at the adaptation period did not show a difference in the combined experimental model (Supplemental Table 2 (Table S2), $P > 0.3$).

The effects of in vivo ELF EMF exposure were detected by the in vitro β-adrenoceptor functional model. The effect of 10 μT ELF EMF in vivo treatment on the NE-activated β-adrenoceptor mediated intracellular level of cAMP is depicted in Figure 3.

Compared to the untreated response of the adaptation period, the β-adrenoceptor mediated cAMP levels were decreased ($P < 0.001$) during the time of the ELF EMF treatment (Figure 3).

Characterization of ELF EMF Treatment on NE-activated β-adrenoceptor Functions Over Time

The effect of ELF EMF was significant over time compared to the control group during wk 3–5 (differences: wk 3: 4.04 with 95% CI of [0.66; 7.41]; wk 4: 8.29 with 95% CI of [5.61; 10.97]; wk 5: 5.66 with 95% CI of [2.08; 9.24] measured in nmol cAMP/mL RBC SUSP; $P < 0.01$; Supplemental Table 3 (Table S3)). The values of the control group did not change (16.7 nmol cAMP/mL RBC SUSP with the 95% CI of [15.3; 18.2]) throughout the whole experiment (Figure 4; Supplemental Table 3 (Table S3)).

The cAMP level of subjects in the ELF EMF treated group had a decrease ($P < 0.001$) of 2.6 nmol/mL RBC SUSP per wk (95% CI: [−2.8; −2.4]) in the treatment period, while there was an increase (1.5 nmol cAMP/mL RBC SUSP per wk; 95% CI: [1.2; 1.9]; $P < 0.001$) in the regeneration period (Figure 4; Supplemental Table 3 (Table S3)). At the intercept of the 2 fitted linear pieces (wk 4), the mean cAMP level was

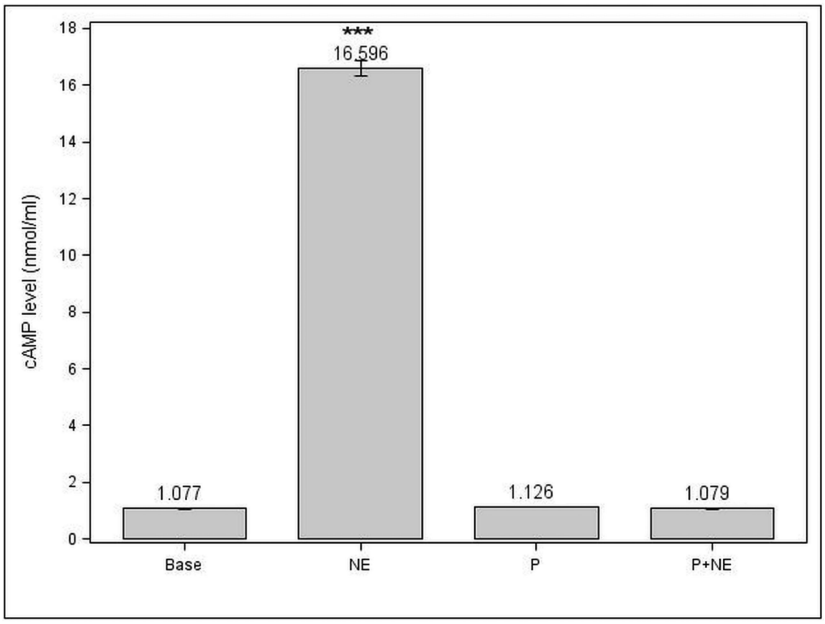

Figure 2. Verification of in vitro experimental model for NE-activated β-adrenoceptor function at adaptation (wk 1, no ELF EMF exposure). Mean (cAMP levels) ± SE at adaptation (wk 1). Mean and SE are calculated on n = 40 animals (treated group from wk 2). Notation: ELF, extremely low frequency; EMF, electromagnetic field; cAMP: 3′5′-cyclic-adenosine-monophosphate; Base, baseline; NE, norepinephrine; P, propranolol; nmol/mL, nmol cAMP/mL RBC SUSP; RBC, red blood cell; SUSP, suspension. ***indicates difference between pairs of NE and all other groups with Type I error $\alpha = 0.001$.

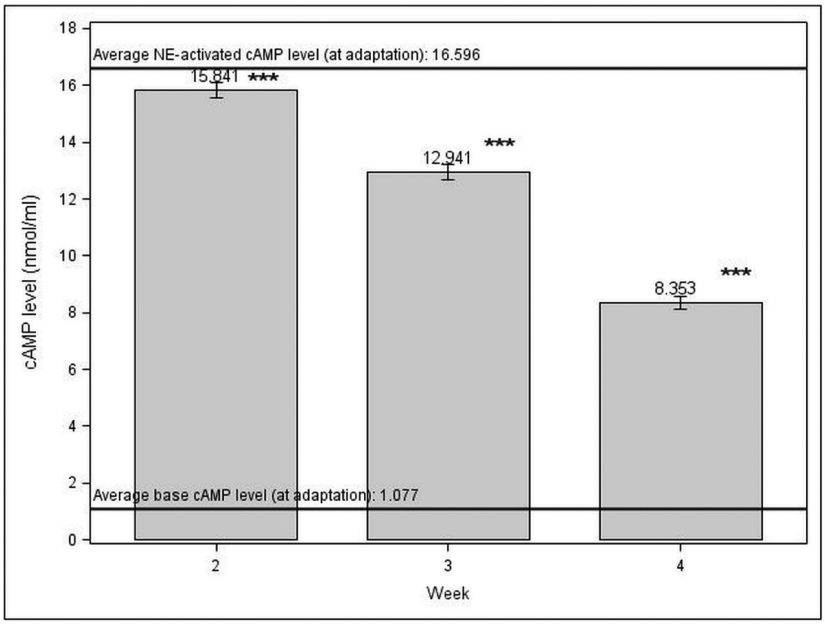

Figure 3. Effects of in vivo ELF EMF treatment on the NE-activated β-adrenoceptor function. Mean NE-activated cAMP levels with SE during treatment period (wk 2–4). Reference lines represent mean cAMP (nmol/mL RBC SUSP) level at the adaptation period (wk 1) for baseline (lower) and NE-activation (upper). Notation: ELF, extremely low frequency; EMF, electromagnetic field; NE, norepinephrine; base, baseline; cAMP: 3′5′-cyclic-adenosine-monophosphate, RBC, red blood cell; SUSP, suspension. ***indicates difference from NE-activated cAMP level at the adaptation (wk 1) with Type I error $\alpha = 0.001$.

9.6 nmol/mL RBC SUSP in the treated group, which was 7.1 nmol/mL RBC SUSP (95% CI: [-8.6; -5.5]) less than that of the control group (16.7 nmol/mL RBC SUSP, Figure 4; Supplemental Table 3 (Table S3)). As 60% of the variation of cAMP levels comes from individual characteristics of turkeys, 40% is from within subject effects.

The Reversible Nature of the Biological Experimental Model

After the treatment with ELF EMF, the main question was whether or not the biological system (NE-activated β-adrenergic receptor function) was capable of returning to the starting state. Furthermore,

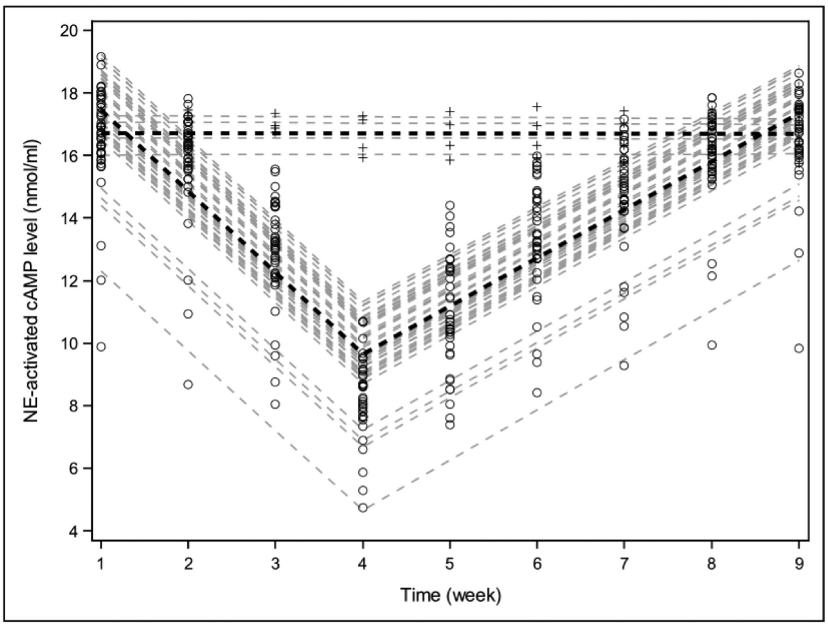

Figure 4. Fitted analysis model for the complete experiment. Result of piecewise linear mixed model. Dashed thick, black lines represent predicted means. Dashed dark grey lines show individual trajectories. Notations: + control (n = 4), o treated (n = 40) subjects, cAMP, 3′5′-cyclic-adenosine-monophosphate; NE, norepinephrine; nmol/mL, nmol cAMP/mL RBC SUSP; RBC, red blood cell; SUSP, suspension.

a relevant question was whether, and if so, how this capacity was modified during the treatment. For this reason, the NE-activated β-adrenoceptor function was investigated weekly, over the regeneration period. The alterations of the β-adrenoceptor function are demonstrated during the experimental period (adaptation, ELF EMF treatment, regeneration) in Figure 4 and Supplemental Table 3 (Table S3). The measured NE-activated levels of cAMP were in compliance with the normal parameters at the end of the adaptation period. The piecewise linear mixed model revealed a decreased effectiveness of β-adrenoceptors, as the NE-activated cAMP level was getting lower and lower during the treatment with ELF EMF. After the end of the ELF EMF treatment, the β-adrenoceptor functions returned to the starting state in 5 wk. As mentioned above, the recovery (increase) of β-adrenoceptor functions in the treated group was slower in the regeneration period (slope of cAMP: 1.5 nmol/mL RBC SUSP) compared to the rate of decrease (slope of cAMP: −2.6 nmol/mL RBC SUSP) in the treatment period (Figure 4; Supplemental Table 3 (Table S3)). The treated group did not differ from the control at the end of the regeneration period (wks 6 to 9, $P \geq 0.15$; Supplemental Table 3 (Table S3)).

DISCUSSION

The effect of ELF EMF has been investigated from many aspects previously, using repeated measurements to characterize the pattern of the examined indicator over time: breeding and migrating behavioral aspects of birds (Hanowski et al., 1996), hematological parameters in mice (Bonhomme-Faivre et al., 2004), and melatonin levels in calves (Kolbabová et al., 2015). However, behavioral patterns defined by receptor functions using mixed models have been rarely considered. Repeated measures experiments have been used commonly in animal, plant, and human research for several decades, and computing methodologies have been available to analyze them effectively and efficiently in the last few decades (Littell et al., 1998).

In our study, we aimed to confirm the effect of in vivo 10 μT ELF EMF treatment on the NE-activated β-adrenoceptor function of physiological processes as cellular mechanisms. The interpretation of our results was verified by parallel and repeated measurements. There were no missing values in our measured data, although mixed models can be used on longitudinal data with missing values (Sapp et al., 2004).

During the experiment, we monitored the serum toxicological parameters (content of hemoglobin, SGOT, SGPT, AP, γGT, LDH), in which there were no differences compared to the normal ranges. According to our observations, the turkeys showed depression-related behavior (less scratching and picking, sleeping more, tucking their heads under their wings) during the ELF EMF treatment. To prove the involvement of NE-dependent β-adrenoceptor function (Takenaka et al., 2012; Guereschi et al., 2013; Zhang et al., 2013; Takenaka et al., 2016) regarding the physiological background of disinterested behavior, an experimental model (Figure 1) was performed (Anisman and Zacharko, 1990; Ressler and Nemeroff, 2001). The in vivo exposition model of turkeys was carried out with a technologically and methodologically standardized in vitro model (Figure 2). In line with the literature (Davoren and Sutherland, 1963; Yin et al.,

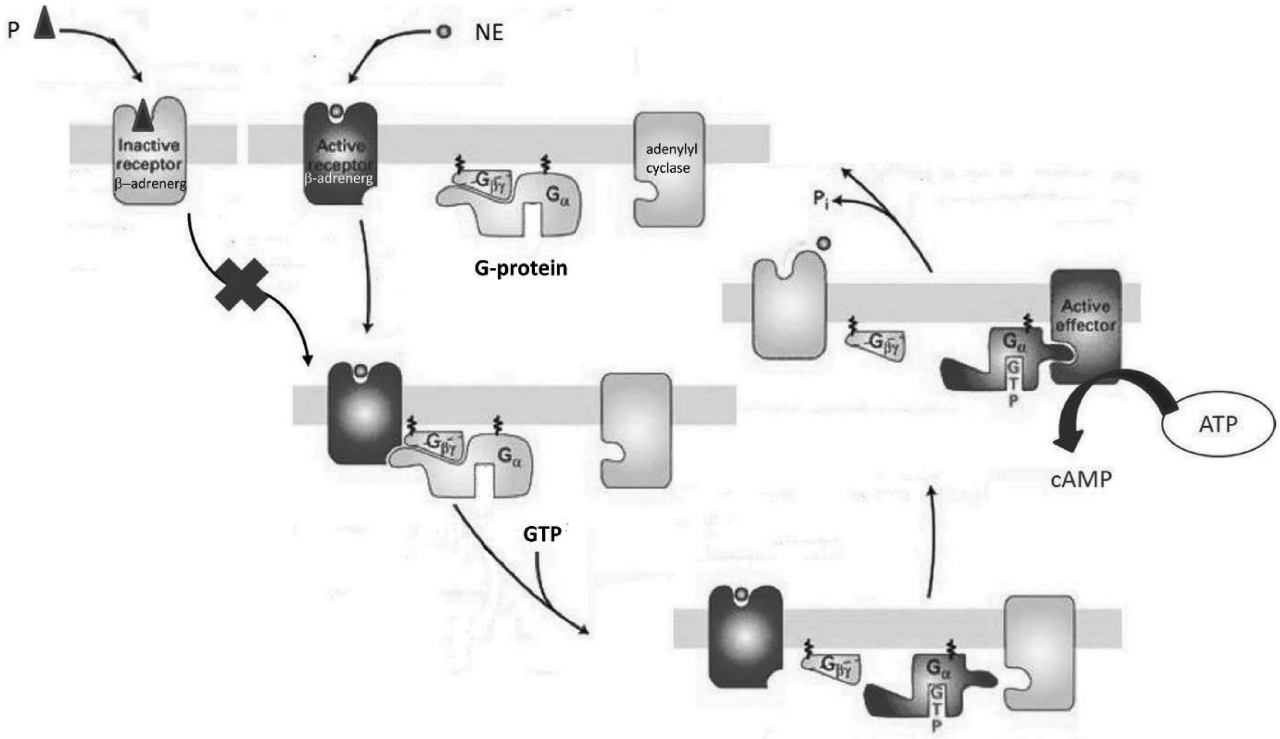

Figure 5. NE-activated β-adrenoceptor cycle (attractor). Notations: P, propranolol; NE, norepinephrine; GTP, guanosine triphosphate; ATP, adenosine triphosphate; cAMP, 3′5′-cyclic-adenosine-monophosphate. Figure modified after J. Hernandez and Wachter and Gilbert (Wachter and Gilbert, 2012).

2016), intense effects were observed on NE-activated β-adrenergic receptors (Figure 2), which verified the model's applicability in the study of functional cycling of NE-activated β-adrenoceptor (Figure 5).

Alteration of behavioral patterns by a 3-wk-long 10 μT ELF EMF treatment was explored by statistical analyses, which were correlated with the decrease of NE-activated β-adrenoceptor function (Figure 3; objective 1). We confirm previous findings, that the decreased NE-activated β-adrenoceptor function has an important role in the formation of emotional disinterest and depression (Morilak and Frazer, 2004; Moret and Briley, 2011).

The difference in sample size (4 vs. 40) of the investigated groups, could cause biased estimates. Different statistical methods were applied that basically had the same result concerning the significant effect of ELF EMF over time (Figure 4; objective 2).

Additionally, fitting piecewise linear mixed model to the data, the treatment and regeneration periods could be characterized separately, which reflected a sharp linear reduction in NE-activated β-adrenoceptor function in the treatment period, and then a remarkable—likewise linear—growth in the regeneration period (Figure 4; objectives 2–3). In another study, piecewise linear model was also applied on poultry-related data (Zuidhof et al., 2009).

Besides the statistically verified effect of ELF EMF, it was important to investigate another interesting phenomenon. Namely, whether the biological cycle (which determines receptor function) was able to return spontaneously—supported by the homeostatic regulatory complex (Holling, 1973; Albert et al., 2000)—to its starting condition, after the treatment period. Therefore, the chosen indicators of our experimental protocol were followed up in the regeneration period after the treatment period. Figure 4 and Supplemental Table 3 (Table S3) depict the success of the regeneration.

In our experiment (neuro-endocrine-immune regulation), turkeys are homeostatic complexities, that are defined as living biological system networks (Barabasi and Albert, 1999). Elementary attractors of complex networks respond to the changing surroundings (assumptions) by performing various operation characteristics (Luque and Ferrera, 2000). This was proved by the biological response of turkeys to ELF EMF exposition. The level of the indicator (intracellular cAMP) decreased and so represented loss of function under the effect of ELF EMF. However, the examined NE-dependent β-adrenoceptor attractor was converged to the starting condition in the regeneration period by the support of the in vivo homeostatic complex network. At the same time, data of the control system reflect no induced alteration. Little disturbance was induced in the NE-activated β-adrenoceptor attractor by the chronic effect of ELF EMF treatment, which was proved by the reversibility. It is important to note that the reversible disturbance of the NE-dependent β-adrenoceptor cycle—caused by a 3-wk-long treatment period—was able to be compensated for by the

homeostatic complexity during the 5 wk in the regeneration period (objective 3). Such extensive in vivo and in vitro experiments combined with sophisticated analysis methods could be useful in environmental studies, may help prevent harmful environmental effects, and could affect public health and the economy.

SUPPLEMENTARY DATA

Supplementary data are available at *PSCIEN* online.

Table S1. Descriptive statistics of cAMP levels (NE-activated β-adrenoceptor function) by treatment groups over time.

Table S2. Comparison of cAMP levels (NE-activated β-adrenoceptor function) of control animals in the adaptation period.

Table S3. Estimations of the marginal model (a) and of the piecewise linear mixed model (b) on the cAMP levels (NE-activated β-adrenoceptor function).

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ANIMAL SUBJECTS RESEARCH

The animal care and procedures were carried out in accordance with the European Communities Council Directive 86/609/EEC. Formal approval to conduct the experiments was granted in advance by the Animal Experimentation Committee at the University of Szeged, Hungary.

STATEMENT OF INFORMED CONSENT

The manuscript does not contain clinical studies or patient data.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Albert, R., H. Jeong, and A. L. Barabasi. 2000. Error and attack tolerance of complex networks. Nature 406:378–382.

Anisman, H., and R. M. Zacharko. 1990. Multiple neurochemical and behavioral consequences of stressors: Implications for depression. Pharmacol. Ther. 46:119–136.

Balmori, A. 2009. Electromagnetic pollution from phone masts. Effects on wildlife. Pathophysiology 16:191–199.

Barabasi, A. L., and R. Albert. 1999. Emergence of scaling in random networks. Science 286:509–512.

Bonhomme-Faivre, L., C Slama, M. L Tanguy, R Santini, Y Bezie, S Marion, L Bottius, N. L Pham, and S Orbach-Arbouys. 2004. Hematologic and cortisol alterations observed in young mice placed in front of a color television screen. Electromagn. Biol. Med. 23:19–27.

Cifra, M., J. Z. Fields, and A. Farhadi. 2011. Electromagnetic cellular interactions. Progr. Biophys. Mol. Biol. 105:223–246.

Curran, P. J., K. Obeidat, and D. Losardo. 2010. Twelve frequently asked questions about growth curve modeling. J. Cogn. Dev. 11:121–136.

Davoren, P. R., and E. W. Sutherland. 1963. The effect of L-epinephrine and other agents on the synthesis and release of adenosine 3', 5'-phosphate by whole pigeon erythrocytes. J. Biol. Chem. 238:3009–3015.

Dickey, D. A. 2008. PROC MIXED: Underlying ideas with examples. Paper 374, SAS Global Forum.

Feychting, M., A. Ahlborn, and L. Kheifets. 2005. EMF and health. Pages 165–189 in Annual Review of Public Health Annual Reviews, Palo Alto.

Ficken, R. W., and M. S. Ficken. 1966. A review of some aspects of avian field ethology. Auk 83:637–661.

Funk, R. H. W., T. Monsees, and N. Ozkucur. 2009. Electromagnetic effects - From cell biology to medicine. Progr. Histochem. Cytochem. 43:177–264.

Gardner, J. D., R. S. Mensh, D. R. Kiino, and G. D. Aurbach. 1975. Effects of β-adrenergic catecholamines on potassium transport in turkey erythrocytes. J. Biol. Chem. 250: 1155–1163.

Guereschi, M. G., L. P. Araujo, J. T. Maricato, M. C. Takenaka, V. M. Nascimento, B. C. Vivanco, V. O. Reis, A. C. Keller, P. C. Brum, and A. S. Basso. 2013. Beta2-adrenergic receptor signaling in CD4(+) Foxp3(+) regulatory T cells enhances their suppressive function in a PKA-dependent manner. Eur. J. Immunol. 43:1001–1012.

Hamer, R. M., and P. M. Simpson. 2000. Mixed-up mixed models: things that look like they should work but don't, and things that look like they shouldn't work but do. Paper 20-25, Twenty-fifth Annual SAS Users Group International Conference. http://www2.sas.com/proceedings/sugi25/25/aa/25p020.pdf

Hanowski, J. M., G. G. Niemi, and J. G. Blake. 1996. Response of breeding and migrating birds to extremely low frequency electromagnetic fields. Ecol. Appl. 6:910–919.

Hayat, M. J., and H. Hedlin. 2012. Modern statistical modeling approaches for analyzing repeated-measures data. Nurs. Res. 61:188. doi 10.1097/NNR.0b013e31824f5f58; 10.1097/NNR.0b013e31824f5f58

Holling, C. S. 1973. Resilience and stability of ecological systems. Ann. Rev. Ecol. Syst. 4:1–23.

James, S. R., C. Vaziri, T. R. Walker, G. Milligan, and C. P. Downes. 1994. The turkey erythrocyte β-adrenergic receptor couples to both adenylate cyclase and phospholipase C via distinct G-protein α subunits. Biochem. J. 304:359–364.

Kavet, R. I., and R. S. Banks. 1986. Emerging issues in extremely-low-frequency electric and magnetic-field health research. Env. Res. 39:386–404.

Kolbabová, T., E. P. Malkemper, L. Bartoš, J. Vanderstraeten, M. Turčáni, and H. Burda. 2015. Effect of exposure to extremely low frequency magnetic fields on melatonin levels in calves is seasonally dependent. Sci. Rep. 5. doi 10.1038/srep14206

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216.

Littell, R. C., J. Pendergast, and R. Natarajan. 2000. Modelling covariance structure in the analysis of repeated measures data. Stat. Med. 19:1793.

Luque, B., and A. Ferrera. 2000. Measuring mutual information in random Boolean networks. Complex Syst. 12:241–252.

Matsuzawa, T., Y. Hayashi, M. Nomura, T. Unno, T. Igarashi, T. Furuya, K. Sekita, A. Ono, and Y. Kurokawa. 1997. A survey of the values of clinical chemistry parameters obtained for a common rat blood sample in ninety-eight Japanese laboratories. J. Toxicol. Sci. 22:25–44.

Moret, C., and M. Briley. 2011. The importance of norepinephrine in depression. Neuropsych. Dis. Treat. 7:9–13.

Morilak, D. A., and A. Frazer. 2004. Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. Int. J. Neuropsychopharmacol. 7:193–218.

Nagyeri, G., Z. Valkusz, M. Radacs, T. Ocsko, P. Hausinger, M. Laszlo, F. A. Laszlo, A. Juhasz, J. Julesz, and M. Galfi. 2012. Behavioral and endocrine effects of chronic exposure to low doses of chlorobenzenes in Wistar rats. Neurotoxicol. and Teratol. 34:9–19.

Naumova, E. N., A. Must, and N. M. Laird. 2001. Tutorial in Biostatistics: Evaluating the impact of 'critical periods' in longitudinal studies of growth using piecewise mixed effects models. Int. J. Epidemiol. 30:1332. doi 10.1093/ije/30.6.1332

O'Halloran, J., P. F. Duggan, and A. A. Myers. 1987. Determination of hemoglobin in birds by a modified alkaline hematin (D-575) method. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 86:701–704.

Palanza, P., L. Gioiosa, F. S. vom Saal, and S. Parmigiani. 2008. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Env. Res. 108:150–157.

Remaley, A. T., and P. Wilding. 1989. Macroenzymes: biochemical characterization, clinical significance, and laboratory detection. Clin. Chem. 35:2261–2270.

Ressler, K. J., and C. B. Nemeroff. 1999. Role of norepinephrine in the pathophysiology and treatment of mood disorders. Biol. Psychiatr. 46:1219–1233.

Ressler, K. J., and C. B. Nemeroff. 2000. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depression Anxiety 12:2–19.

Ressler, K. J., and C. B. Nemeroff. 2001. Role of norepinephrine in the pathophysiology of neuropsychiatric disorders. CNS Spectrums 6:663–670.

Rudolph, S. A., and P. Greengard. 1974. Regulation of protein phosphorylation and membrane permeability by β adrenergic agents and cyclic adenosine 3':5' monophosphate in the avian erythrocyte. J. Biol. Chem. 249:5684–5687.

Santini, M. T., G. Rainaldi, and P. L. Indovina. 2009. Cellular effects of extremely low frequency (ELF) electromagnetic fields. Int. J. Radiat. Biol. 85:294–313.

Sapp, R. L., R. Rekaya, I. Misztal, and T. Wing. 2004. Male and female fertility and hatchability in chickens: A longitudinal mixed model approach. Poult. Sci. 83:1253–1259.

SAS. 2011. SAS/STAT 9.3 User's Guide. SAS Institute Inc., Cary, NC.

Singer, J., and J. Willett. 2003. Applied longitudinal data analysis: Modeling change and event occurrence. Oxford University Press, New York, NY.

Sutherland, E. W., and G. A. Robison. 1966. The role of cyclic-3',5'-AMP in responses to catecholamines and other hormones. Pharmacol. Rev. 18:145–161.

Takenaka, A., S. Nakamura, F. Mitsunaga, M. Inoue-Murayama, T. Udono, and B. Suryobroto. 2012. Human-Specific SNP in Obesity Genes, Adrenergic Receptor Beta2 (ADRB2), Beta3 (ADRB3), and PPAR gamma 2 (PPARG), during Primate Evolution. PLoS ONE 7:10. doi 10.1371/journal.pone.0043461

Takenaka, M. C., L. P. Araujo, J. T. Maricato, V. M. Nascimento, M. G. Guereschi, R. M. Rezende, F. J. Quintana, and A. S. Basso. 2016. Norepinephrine Controls Effector T Cell Differentiation through beta 2-Adrenergic Receptor-Mediated Inhibition of NF-kappa B and AP-1 in Dendritic Cells. J. Immunol. 196:637–644.

Tomas, G., E. Barba, S. Merino, and J. Martinez. 2012. Clutch size and egg volume in great tits (Parus major) increase under low intensity electromagnetic fields: A long-term field study. Env. Res. 118:40–46.

Uhde, T. W., J. P. Boulenger, R. M. Post, L. J. Siever, B. J. Vittone, D. C. Jimerson, and P. P. Roybyrne. 1984. Fear and anxiety - relationship to noradrenergic function. Psychopathology 17: 8–23.

Wachter, S. B., and E. M. Gilbert. 2012. Beta-adrenergic receptors, from their discovery and characterization through their manipulation to beneficial clinical application. Cardiology 122: 104–112.

Yin, X., L. Zhou, F. Han, J. Han, Y. Zhang, Z. Sun, W. Zhao, Z. Wang, and L. Zheng. 2016. Beta-adrenoceptor activation by norepinephrine enhances lipopolysaccharide-induced matrix metalloproteinase-9 expression through the ERK/JNK-c-Fos pathway in human THP-1 cells. J. Atheroscler. Thromb.

Zeger, S. L., K. Y. Liang, and P. S. Albert. 1988. Models for longitudinal data - a generalized estimating equation approach. Biometrics 44:1049–1060.

Zhang, L., M. Ouyang, C. R. Ganellin, and S. A. Thomas. 2013. The slow afterhyperpolarization: a target of beta(1)-adrenergic signaling in hippocampus-dependent memory retrieval. J. Neurosci. 33:5006–5016.

Zuidhof, M. J., M. Betti, D. R. Korver, F. I. L. Hernandez, B. L. Schneider, V. L. Carney, and R. A. Renema. 2009. Omega-3-enriched broiler meat: 1. Optimization of a production system. Poult. Sci. 88:1108–1120.