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Weak radiofrequency fields affect the insect circadian clock

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It is known that the circadian clock in *Drosophila* can be sensitive to static magnetic fields (MFs). Man-made radiofrequency (RF) electromagnetic fields have been shown to have effects on animal orientation responses at remarkably weak intensities in the nanotesla range. Here, we tested if weak broadband RF fields also affect the circadian rhythm of the German cockroach (*Blatella germanica*). We observed that static MFs slow down the cockroach clock rhythm under dim UV light, consistent with results on the *Drosophila* circadian clock. Remarkably, 300 times weaker RF fields likewise slowed down the cockroach clock in a near-zero static magnetic field. This demonstrates that the internal clock of organisms can be sensitive to weak RF fields, consequently opening the possibility of an influence of man-made RF fields on many clock-dependent events in living systems.

1. Introduction

Radiofrequency (RF) fields accompanying modern man are suspected to interfere with biological processes and have been extensively examined (reviewed e.g. in [1,2]). The impact of electromagnetic noise on animal magnetoreception is well documented and the compass sense of birds [3–7], mice [8] and arthropods [9,10] was lost or biased [11] if animals were exposed to RF. RF fields have been suggested to interfere specifically with radical-pair (RP) magnetoreception mechanism [3,12–14], and it has been suggested that key steps in magnetic field (MF) reception may be mediated by Cryptochrome protein (Cry) ([12], reviewed in [15]). Gene silencing identified *Blatella germanica* Cry as essential for detection of directional changes in MFs [16]. Cry is known to be a component of the biological clock system. Two independent studies have shown that static MFs with intensities comparable to the Earth's MF changed the circadian rhythm in *Drosophila* [17,18]. The MF effect was dependent on functional *cry* gene and the wavelength of light. Aside from magnetoreception and the circadian clock, Cry is involved in several cellular and organismal functions [19,20] and pathologies [21,22]. The possibility that MF and especially widespread weak RFs interfere with Cry cellular tasks in general is both intriguing and pungent. Here, we set out to investigate if the circadian rhythm of *Blatella* is affected by MFs and weak broadband RF fields.

2. Results and discussion

To explore a role of magnetic and RF fields on the circadian clock, we investigated the free-running rhythm of locomotor activity of the German cockroach (*Blatella germanica*) in darkness and two intensities of UV 365 nm light combined with three intensities of static MF and three intensities of RF fields.

First, two intensities of UV 365 nm constant light which reliably lengthen but still not abolish the free-running period were set: dimmer: 1.7×10^{-6} Wcm^{-2} and

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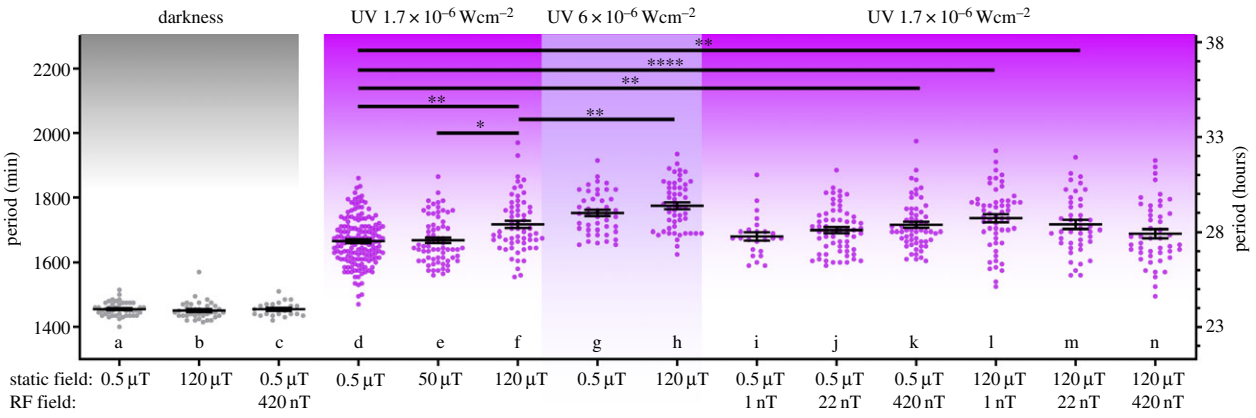


Figure 1. Periods of locomotor activity under permanent darkness or UV light 365 nm. The light lengthens the period proportionally to its intensity as well as static 120 µT and 420 nT RF field separately do. Light is necessary for both MF and RF fields effects. The threshold is between 50 and 120 µT for MF and between 22 and 420 nT for RF. Mean with s.e.m. are figured, Tukey's multiple comparisons test used, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, for all significant differences, $****p < 0.0001$, see electronic supplementary material, figure S1. (Online version in colour.)

brighter: 6×10^{-6} Wcm^{-2}. When keeping the MF at both 0.5 µT and 120 µT, the observed lengthening of the free-running period in *Blatella* reflects the intensity of light (electronic supplementary material, figure S1 and figure 1f,h) and is consistent with *D. melanogaster* results [17].

As a second step, the role of a static MF was tested. In the dimmer UV light 1.7×10^{-6} Wcm^{-2}, we used three intensities: near-zero 0.5 µT (figure 1d), Earth-like 50 µT (figure 1e) and hypermagnetic 120 µT (figure 1f). While significant lengthening of the period was evident when the MF was strengthened from 50 µT to 120 µT, no change of the period occurred between 0.5 and 50 µT (figure 1). Under brighter light 6×10^{-6} Wcm^{-2}, lengthening of the period between 0.5 µT (figure 1g) and 120 µT (figure 1h) was not significant, likely to be due to getting close to saturation limit. The system clearly needs the light to become magnetosensitive as no change of the rhythm was detected in darkness. The static MF effects observed in our study are clearly dependent on the presence, intensity and wavelength of light—all in line with two *Drosophila* reports from Yoshii *et al*. [17] and Fedele *et al*. [18].

In our system, the application of a 120 µT MF slowed down the circadian rhythm compared to both 0.5 and 50 µT fields and resembles the impact of UV light (figure 1). However, the same-treatment under 505 nm green light 4.5×10^{-6} Wcm^{-2} shortened the period of the rhythm (electronic supplementary material, figure S2). Antagonistic effects of UV-blue versus blue-green parts of the spectra have been reported several times in animal magnetoreception (rev. e.g. in [23]) and provide the most likely explanation for ambiguous or opposite responses of *Drosophila* clock system to MFs [17,18]. The nature of this antagonism is beyond the scope of our work, but is clearly in line with involvement of light-dependent magnetoreception in animal clock systems.

Having established that static MF effects occur on the cockroach circadian clock, we turn to studying the effects of weak RF MFs. In this study, we choose to apply RF broadband noise of different intensities, because it has been argued that such fields may be a promising diagnostic test for the presence of an underlying RP mechanism [13].

Application of RF broadband noise in near-zero MF and dim 1.7×10^{-6} Wcm^{-2} UV light did not have a significant effect for a 1 nT (figure 1i) or 22 nT RF field (figure 1j), but reached significant level of slowing the rhythm for a 420 nT RF field (figure 1k), when compared with the near-zero MF dim UV light condition without an RF field (figure 1d). Therefore, the effect of the 400 nT RF field (figure 1k) was statistically indistinguishable from the effect of the 120 µT static MF (figure 1f). In contrast to the effects under dim UV light, no effect of a 420 nT broadband RF field could be observed in darkness (figure 1c), just as no effects of a 120 µT could be observed in darkness (figure 1b). This suggests a similar mechanism of action of the RF broadband field to the static MF. However, it is noteworthy that the intensity of the RF field required to slow down the circadian clock was much lower than that required for a static MF to do the same. The threshold for static MF field effects to occur lies between 50 and 120 µT, indicating that the clock system is between 100 and 300 times more sensitive to RF field than to static MF.

When RF broadband noise combined with 120 µT MF and dim 1.7×10^{-6} Wcm^{-2} UV light were applied, no significant effects on the circadian clock were seen for 1 nT (figure 1l) and 22 nT (figure 1m) RF fields again. Nevertheless, a subtractive relationship may be hypothesized for RFs and MF if applied simultaneously since a combination of maximal RF 420 nT and maximal MF 120 µT (figure 1n) did not differ from control near-zero condition (figure 1d). Such interference between MFs and RFs has its parallel in experiments on animal orientation discussed above but its physical nature, likely to be dependent on the (an)isotropy of hyperfine interactions within RP (P Hore 2018, personal communication), is beyond the scope of the work. The observed threshold of broadband RF field effects between 22 and 420 nT should be considered dependent on background MF and light intensities, hence potentially, even more sensitive. Should the sensitivity of the clock system be as high as to about tens of nT of cumulative intensity, biological experiments in standard laboratories or incubators may face biasing problems, because background RF intensity may often reach this level (electronic supplementary material, figure S3; 30 nT for bench measurement outside the shielded chamber).

3. Conclusion

Our data show that the circadian rhythm of the insect species *B. germanica* is sensitive to both static MF and RF fields in a

light-dependent manner. While a number of studies have shown physiological effects of weak RF fields as mentioned in the Introduction, all of these effects were observed on magnetic orientation responses of the respective animals and one might therefore argue that the effects of RF fields may be limited to highly specialized responses of navigating animals. Having no ambitions to explain the nature of observed phenomena, our study provides evidence that weak broadband RF noise impacts the insect clock system which turned out to be even more sensitive to RF than to static MFs. Studies of free-running period are always performed under artificial conditions devoid of any natural synchronizing inputs (Zeitgebers) which limits all conclusions referring to normal conditions on the Earth. Nevertheless, since the number of circadian clock-dependent health problems grows in population and since urban areas are polluted by RF noise even more than it was used in this study (e.g. [6]), the evidence of interference between RF and clock controlling systems is worth of concern and deep investigation.

4. Material and methods

4.1. Testing scheme

Adult German cockroaches immobilized by CO_2 were individually and regardless of sex transferred into glass Petri dishes on a layer of transparent agarose gel. Forty dishes were placed on a glass pane on a wooden table into an electromagnetically shielded chamber illuminated from above and monitored by a camera from below. Silhouettes of cockroaches were captured every 5 min for 14 days and free-running movement rhythms were investigated. Thermal (23.8°C ± 0.6°C), light and magnetic conditions were held constant for the entire testing period and were checked prior to and after the experiment. Different experimental conditions alternated (see table of primary data available online). Experimenter and data evaluator were always blind as to what type of RF field and MF were set.

4.2. Light conditions

Two intensities of UV light (365 nm) and three intensities of green light (505 nm) and darkness were generated by LED lamps. Petri dishes had opaque white walls and a light-dispersing sheet of filter paper covered them.

4.3. Magnetic conditions

Three static MFs intensities were set: total vector 0.5 µT ± 0.4 µT (residual field, no current sent to coils), total vectors 50 µT and 120 µT (space deviation ± 0.5 µT) and inclinations 45° (chosen for technical reasons) were generated by three-dimensional Merritt coils (2.5 × 2.5 × 2.5 m) located inside the chamber and fed by custom-made, computer-controlled power supplies.

4.4. RF conditions

Four experimental intensities of the broad band RFs noise (of 0, 1, 22 and 420 nT) were generated by a loop antenna constructed as a single horizontal winding of coaxial cable around the walls of the chamber in the plane of the testing table. The shield of the coaxial cable was removed opposite the feed.

4.5. Evaluation and statistics

For motor activity detection, 4032 samples were analysed and the number of body shifts greater than 1 cm was calculated automatically using Matlab based custom-made image analysis software RoachLab. Actograms and their statistical significances were analysed by the Lomb–Scargle method plugin ActoJ of ImageJ software (1.49v; NIH). All tested conditions were visualized and statistically evaluated by ANOVA and Tukey's multiple comparisons test (GraphPad Prism 7.04). Only clearly rhythmic animals with statistical significance PN > 20 were scored and analysed (percentage of rhythmic animals is given in electronic supplementary material, figures S1 and S2).

Data accessibility. Complete list of primary data is available online at: https://is.muni.cz/www/vacha/supplementary_materials_blatella_rhythms/Blatella_rhythms_primary_data.xls.

Competing interests. We declare we have no competing interests.

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