

This is the peer reviewed version of the following article: Todorović D, Ilijin L, Mrdaković M, Vlahović M, Grčić A, Petković B, Perić-Mataruga V. The impact of chronic exposure to a magnetic field on energy metabolism and locomotion of *Blaptica dubia*. *Int J Radiat Biol.* 2020;96(8):1076-83. <https://doi.org/10.1080/09553002.2020.1770360>.



© 2020 Taylor & Francis Group LLC.

**The impact of chronic exposure to a magnetic field on energy metabolism and locomotion
of *Blaptica dubia***

Dajana Todorović¹ (0000-0003-1796-9312), Larisa Ilijin¹ (0000-0003-1728-6140), Marija
Mrdaković¹ (0000-0003-3946-5828), Milena Vlahović¹ (0000-0002-7815-091X), Anja Grčić¹
(0000-0001-9306-0691), Branka Petković² (0000-0001-7817-4092), Vesna Perić-Mataruga¹
(0000-0002-2899-7418)

¹Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša
Stanković" - National Institute of Republic of Serbia, University of Belgrade, Despot Stefan
Blvd. 142, 11060 Belgrade, Serbia

²Department of Neurophysiology, Institute for Biological Research "Siniša Stanković" - National
Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blvd. 142, 11060
Belgrade, Serbia

Corresponding author: Dajana Todorović, PhD, Department of Insect Physiology and
Biochemistry, Institute for Biological Research "Siniša Stanković" - National Institute of
Republic of Serbia, University of Belgrade, Despot Stefan Blvd. 142, 11060 Belgrade, Serbia.
Tel.: +381 11 2078 350. Fax: +381 11 2761 433. E-mail: dajana@ibiss.bg.ac.rs

Abstract

Purpose: This study deals with a comparative analysis of the effects of chronic exposure to a static magnetic field (SMF) and an extremely low frequency magnetic field (ELF MF) in *Blaptica dubia* nymphs. The outcome of such treatment on insect and fat body mass, glycogen and total lipid content in the fat body and locomotion, as an energy demanding process, were examined.

Materials and methods: One-month-old nymphs of *B. dubia* were exposed to an SMF (110 mT) or ELF MF (50 Hz, 10 mT) for 5 months. Their locomotion was monitored in the "open-field" test for 10 min and expressed as travel distance, time in movement and average speed while in motion. After that, fat body mass and content of its main components (glycogen and total lipids) were determined. Nymph body mass was also estimated after 1 and 5 months of MF treatment.

Results: Chronic exposure to the SMF and ELF MF decreased nymph body mass and glycogen content in the fat body, but increased all examined parameters of locomotion. In addition, chronic SMF treatment elevated total lipid content in the fat body, while chronic ELF MF treatment reduced fat body mass and total lipid content.

Conclusions: These findings indicate that *B. dubia* nymphs are sensitive to the applied MFs and possess different strategies for fuel usage in response to the SMF and ELF MF in order to satisfy increased energy demands and to overcome stressful conditions.

Keywords: magnetic field, fat body, glycogen, total lipids, locomotion, cockroaches

Introduction

Since life on Earth originated in a natural magnetic field (geomagnetic field), magnetic fields (MF) are identified as an important abiotic factor directly affecting all living beings. During evolution, organisms developed numerous adaptations to the natural MF that enabled them to survive in altering environmental conditions (Ghodbane et al. 2013). Due to technological developments and intensive industrialization, the level of magnetic and electromagnetic fields to which living beings are exposed is constantly increasing. Under changing conditions, homeostasis of biological systems may be disturbed, resulting in various disorders. Therefore, research attention is increasingly focused on obtaining better understanding of these issues, primarily to protect human health after the introduction of new technologies in everyday life, industry, transportation system and medicine, including therapeutic uses of MF. The available information about the health effects of these MFs included cancer incidence, haematological, reproductive and developmental changes, as well as neurobehavioral effects are contradictory (Zannella 1997; Leszczynski, 2005; WHO 2006; WHO 2007) and additional research is necessary to infer causal relationships.

The biological effects of artificial MFs have already been studied in many organisms. Numerous findings have indicated that an MF can modulate various aspects of insect physiology and behavior (Stanojević et al. 2005; Starick et al. 2005; Rauš et al. 2009; Savić et al. 2011; Yang et al. 2011; Todorović et al. 2012; Todorović et al. 2013; Dimitrijević et al. 2014; Jankowska et al. 2015; Pavlović et al. 2016; Wyszowska et al. 2016; Sheppard et al. 2017; Xu et al. 2017; Zmejkoski et al. 2017; Maliszewska et al. 2018; Todorović et al. 2019).

Physiological processes and behaviors like reproduction, embryogenesis, metamorphosis, locomotion and flight require a steady supply of energy. Insects have developed special systems

for efficient and rapid use of energy stored in the fat body, as lipid and glycogen depots. Fat body functions are similar to those of the liver and adipocytes in vertebrates. The insect fat body consists of cell aggregates, mainly adipocytes, which take up and store nutrients (Beenackers et al. 1985). The main source for energy demanding physiological processes is trehalose in hemolymph, while precursors for trehalose synthesis in the fat body are its glycogen depots or dietary carbohydrates (Steele 1999). Large reserves of triacylglycerol, derived from dietary fatty acids or from sugars and amino acids released during digestion, are also present in the fat body (Oguri and Steele 2003). Insect energy metabolism is hormonally regulated by adipokinetic hormones (AKHs), biogenic amines (predominantly octopamine) and the insulin-like neurohormone bombyxin (Orchard et al. 1993; Satake et al. 1997; Meyer-Fernandes et al. 2000; Van der Horst et al. 2001; Gäde 2004; Lorenz and Gäde 2009). Increased carbohydrate and lipid metabolism occurs under stress to provide the energy necessary for implementing protective mechanisms. It is known that an MF can change the cell membrane potential and distribution of ions (Hughes et al. 2005). This may lead to modification of biochemical processes involved in the energy status of the cell. Thus, increased glucose and lipid metabolism has been shown in mouse muscle (metabolically active mass) and blood after exposure to different types of MF (Kumosani and Qari 2003; Elferchichi et al. 2010).

Locomotion is defined as a behavioral property of all animals that requires energy expenditure. It could be considered as a reflection of the decision-making process (to walk or not to walk) depending on current status (Martin 2003). Insect locomotion has been studied for over a century in a wide variety of species, revealing generally conserved mechanisms of control (Serway et al. 2009; Borgmann and Büschges 2015; Martin et al. 2015). Important processes in the regulation of motor behavior are initiated by the neuroendocrine system (Orchard et al. 1993;

Osborne 1996; Nässel and Winther 2010). Octopamine is the most frequent insect amine that tunes both visceral and skeletal muscle activities (Roeder 1999) and has a functional role in motor behavior (Pflüger and Duch 2000). Other biogenic amines (dopamine, norepinephrine, serotonin, tyramine) also affect the production of AKHs (Van der Horst et al. 2001) that have a neuromodulatory role and stimulate insect locomotion (Socha et al. 1999). AKHs also have a metabolic role and control release of carbohydrates, lipids and the amino acid proline from fat body to hemolymph during locomotion and flight (Gäde and Auerswald 2003; Lorenz et al. 2004; Gálíková et al. 2015). Altered locomotion is one of the first signs of environmental change and the presence of stressors. Thus, exposure to an MF has been shown to modify movement of different insect species (Todorović et al. 2013; Dimitrijević et al. 2014; Pavlović et al. 2016; Wyszowska et al. 2016; Zmejkoski et al. 2017). Moreover, insects can use the natural MF as a means of navigation and spatial orientation (Banks and Srygley 2003; Klotz and Jander 2003; Riveros and Srygley 2010; Guerra et al. 2014; Xu et al. 2017).

Since environmental factors affect the physiological state and behavior of living organisms (Hansen and Berthelsen 2000), our goal was to investigate if a chronic 5-month exposure of *Blattella germanica* nymphs to an MF modifies insect and fat body mass, glycogen and total lipid content in the fat body, and locomotion as an energy demanding process. The impacts of a static magnetic field (SMF; 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) associated with different sources in industry (welding machines, electric and induction furnaces, electrolytic processes, aluminium production), transportation system (rail transport using magnetic levitation) and medicine (magnetotherapy) (Zannella 1997; Leszczynski, 2005; WHO 2006, WHO 2007) were considered and compared. This study has the practical significance since cockroaches are one of the most prominent pest insects that can be

found in almost every place where people live and work. They are exposed to the same MFs as humans and the obtained data can be extrapolated to estimate risks and benefits of such treatment in humans. Further, high voltage power lines emitting MF are often in the trajectory of many species (insects, birds, etc.), thus studies like this can be a good basis to consider how these high levels of MF might impact on other insects that provide valuable ecosystems services such as key pollinators (honey bees) whose cognitive abilities are crucial in finding food.

Material and methods

Model organisms

B. dubia is a synanthropic, cosmopolitan species that is easily grown in the laboratory. These cockroaches have a high reproductive potential, give birth to live young, and their developmental pattern is known (Alamer 2013; Wu 2013). Nymphs of *B. dubia* undergo seven instars over a period of 6 months before reaching adulthood (Hintze-Podufal and Nierling 1986; Wu 2013). They are immature form that already resembles that of the adults, except for a lack of wings. Adults live up to 2 years. All this together with the long life cycle makes this species highly suitable as a model organism, particularly for the evaluation of chronic effects, in neurophysiological, pharmacological, biomedical and environmental studies. In most magnetobiological experiments, biological objects were exposed to MFs for relatively short period, from less than one hour to a few weeks. There have been few studies on the effects of chronic exposure, such as over a few months, despite some indication that test animals become more sensitive to radiation after such treatment (Levitt and Lai 2010).

The 1-month-old nymphs of *B. dubia* used in the experiment were offspring of male and female from the cockroach laboratory stock of the Department of Insect Physiology and Biochemistry, Institute for Biological Research "Siniša Stanković". They were reared in an

experimental room under constant conditions: temperature ($26 \pm 0.2^\circ\text{C}$), relative humidity (60-70%) and photoperiod (12 h light/12 h dark). Food (ground biscuits, dog chops and fresh apples) for all cockroaches (stock population and experimental individuals) was provided *ad libitum* and changed once a week. The advantage of using nymphs of *B. dubia* as a model in this study is that their development lasts long enough (about 6 months), which allows chronic exposure to MFs of individuals at a specific stage of development and attribution of the outcome of such treatment to changes induced by MFs exclusively during this stage. Adults also live long, but nymphs are younger and their survival rate is higher, which is very important for a chronic study to have a valid number of samples for statistical analysis.

Magnetic field exposure systems

B. dubia nymphs were exposed to an SMF (110 mT) generated by a permanent double U shaped magnet (Model 6002, Raytheon, Waltham, MA) or an ELF MF (50 Hz, 10 mT), generated by an electromagnet, as described in detail in Todorović et al. (2019).

All experiments were performed in a room with an ambient geomagnetic field of approximately 47 μT . A GM05 gaussmeter with a PT 2837 probe (Hirst Magnetic Instruments, Falmouth, UK) was used to measure the MF. Throughout the experiments, the temperature was constantly recorded (Environment Meter (DVM 401) - Velleman, USA). Some slight differences, compared to the control room temperature, as well as the temperature in the permanent magnet ($26 \pm 0.2^\circ\text{C}$), were found in the electromagnet ($26.3 \pm 0.05^\circ\text{C}$).

Experimental procedure

The 1-month-old *B. dubia* nymphs were randomly placed in plastic 3.5 cm diameter Petri dishes (5 individuals per dish) and exposed to the SMF (110 mT; SMF group) or ELF MF (50 Hz, 10 mT; ELF MF group) for 5 months, i.e. until the end of their development. Nymphs of the

control group were submitted to the same experimental procedure but remained outside the reach of the MF. Throughout the experiment, all groups were kept under the same optimal conditions (see above under Model organisms).

After 5 months, locomotion of control and MF exposed individuals was monitored in the "open-field" test for 10 min. Thereafter, they were sacrificed, the fat body was isolated on ice and weighed, and its glycogen and total lipid content determined. Nymph body mass was also estimated 1 and 5 months after onset of the experiment.

Determination of fat body glycogen and total lipid content

Fat body glycogen content was determined in individual nymphs using the anthrone reaction (Wyatt and Kale 1957). Each fat body was placed in 30 volumes of deionized water and homogenized on ice, at the lowest speed (5 x 10 000 rpm). After centrifugation (4°C, 10 000 rpm, 10 min; Eppendorf 5417R, Germany) supernatants were collected. The samples were heated in 30% KOH at 100°C for 15-20 min, centrifuged at room temperature for 8 min at 10 000 rpm and the supernatants collected. Following addition of Na₂SO₄ (200 µl) and 96% ETOH (1.2 ml) and centrifugation (4°C, 14 000 rpm, 10 min), the obtained precipitate (glycogen) was diluted in H₂O₂ (400 µl) and 0.15% anthrone added. After heating at 100°C for 8 min, glycogen content was measured spectrophotometrically at 620 nm (SAFAS, Monaco City, Monaco) and expressed as mg glucose per mg fat body.

Fat body lipid content was measured spectrophotometrically using the vanillin phosphoric acid method (Stone and Mordue 1980). Following addition of H₂SO₄ (500 µl) to fat body homogenates (5 µl) the solution was heated in boiling water for 10 min. After cooling to room temperature the vanillin reagent (20 ml; 85% phosphoric acid and 197.7 mg vanillin in cold

deionized water) was added and absorption measured at 546 nm (SAFAS, Monaco City, Monaco). Total lipid content was expressed as mg of lipid per mg of fat body.

Locomotion monitoring

The nymphs were transferred to 8.5 cm diameter Petri dishes (one individual per dish). A LifeCam VX-6000 (Microsoft, Redmond, WA, USA) positioned above the Petri dishes was used to record locomotion of two individuals simultaneously. The camera was connected to a computer with Any-maze behavioral tracking software (v.4.96, Stoelting Co., Wood Dale, IL, USA). The following parameters of locomotion were analyzed by blinded personnel: travel distance (m), time in movement (s) and average speed while in motion (m/s).

Each nymph was tested only once immediately after transfer to the Petri dish. Locomotion was monitored between 9 a.m. and 1 p.m in the experimental room under the same conditions used for the experiment.

Statistical analysis

Data distribution was initially estimated using the Kolmogorov-Smirnov test. The results for insect and fat body mass, as well as glycogen and total lipid content were normally distributed and were analyzed using one-way analysis of variance (ANOVA) followed by post hoc Fisher's Least Significant Difference (LSD) test. The data for locomotion did not fit a normal distribution and they were analyzed using the Kruskal-Wallis ANOVA followed by the post hoc Mann-Whitney U test. In all cases, the probability $p < 0.05$ was considered as statistically significant.

All analyses were performed with STATISTICA v.7.0 software (StatSoft, Tulsa, OK, USA).

Results

Nymph mass and fat body mass

B. dubia nymph body mass was unchanged after a one-month exposure to the MFs ($F = 0.49$, $df = 2$, $p > 0.05$). In contrast, it was reduced by each five-month MF treatment ($F = 9.44$, $df = 2$, $p < 0.001$) when compared to the control group (Table 1). Although the effect was more pronounced in the SMF group, it was not significantly different from that for the ELF MF group.

Changes in fat body mass of nymphs chronically exposed to the MFs for 5 months were also observed ($F = 8.68$, $df = 2$, $p < 0.01$). Both the SMF and the ELF MF caused decreases in fat body mass, but the stronger effect was statistically significant only for the ELF MF (Table 1).

Glycogen and total lipid content in the fat body

Chronic exposure of *B. dubia* nymphs to the MFs for 5 months affected glycogen content ($F = 28.25$, $df = 2$, $p < 0.001$) and total lipid content ($F = 12.91$, $df = 2$, $p < 0.001$) in the fat body. Thus, glycogen level was significantly reduced in both SMF and ELF MF groups to a similar extent when compared to the control group (Figure 1A). In contrast, total lipid content was significantly increased in the SMF group, but significantly decreased in the ELF MF group in comparison with the control group value (Figure 1B). The total lipid content in the ELF MF group was also significantly lower when compared to the SMF group value.

Locomotion

Altered locomotion of *B. dubia* nymphs was another consequence of chronic exposure to MFs for 5 months (travel distance: $H = 19.66$, $df = 2$, $p < 0.001$; time in movement: $H = 18.50$, $df = 2$, $p < 0.001$; average speed while in motion: $H = 19.73$, $df = 2$, $p < 0.001$). In both SMF and ELF MF groups, all three examined parameters of locomotion were significantly increased

compared values for the control group (Figure 2A, B C). The increases were higher in the ELF MF group, but were not significantly different from values for the SMF group.

Discussion

The main finding of this study is that chronic exposure of *B. dubia* nymphs to the SMF (110 mT) or the ELF MF (50 Hz, 10 mT) for 5 months decreased their body mass and the fat body glycogen content, as well as increasing all examined parameters of locomotion. In addition, the SMF treatment elevated total lipid content in the fat body, while the ELF MF treatment reduced fat body mass and its total lipid content.

The concept of energy reorganization under the pressure of stress postulates energy re-allocation and modulation of energy demands for stress compensatory reactions (Djawdan et al. 1998). The insect store of chemical energy is required for three types of physiological processes: biosynthesis (reproduction, growth, energy deposition, etc.), maintenance of function and structure, and external work (Hill et al. 2012). Acquired energy must be divided optimally among these processes to maintain homeostasis and fitness. Under stressful conditions, the priority is energy expenditure to protect survival, so energy flux is redirected to stress-protective reactions (Kooijman 2000).

The fat body is the center for energy metabolism in insects. Energy reserves are deposited in the form of glycogen (carbohydrate) and neutral lipids (fat). The glycogen polymer can be readily degraded to glucose on demand for use as a glycolytic fuel in the stress state (Arrese and Soulages 2010). In insects lipids are the principal energy substrate for long-term extreme circumstances (Chino 1997). During tethered long lasting flight of *Locusta migratoria*, the level of lipids in hemolymph was elevated about three-fold together with acceleration of the diacylglycerol pool turnover rate (Van der Horst et al. 2001). The energy content of fats is much

greater than that of glycogen. As insect fat reserves contain eight to nine times more energy per unit mass than glycogen reserves (Arrese and Soulages 2010), they play the predominant role in energy compensation in severe stress states (Djawdan et al. 1998). Rall et al. (2010) found that insect feeding was reduced and catabolic processes (due to higher energy demands) in the fat body were turbulent under continual stress conditions. Kooijman (2000) proposed a concept of energy-limited tolerance to stress whereupon more energy is spent on somatic maintenance under stress conditions than in enlarging energy reserves, i.e. energy stores become depleted. Our results showed significantly lower nymph body masses after chronic exposure to the SMF or ELF MF, but lower fat body mass only after chronic exposure to the ELF MF. This suggests that in the SMF group energy influx was probably similar to expenditure and therefore the energy depots were not depleted. On the contrary, in the ELF MF group energy demands (locomotion, higher metabolic rate, reactions of stress-compensation, etc.) exceeded energy intake with the consequent deficit being covered by utilization of fat body mass. This could indicate higher susceptibility of *B. dubia* nymphs to the effects of the ELF MF than the SMF, but further research is needed to confirm this assumption.

In line with the above mentioned are our results regarding the influence of chronic exposure to MFs on fat body glycogen and total lipid content in *B. dubia* nymphs. The glycogen content was decreased regardless of MF type, while total lipid content was greater after SMF treatment, but lower after ELF MF treatment. It seems that the nymphs used different energy resources in response to the SMF (carbohydrates) and ELF MF (carbohydrates and lipids) to satisfy the increased energy demands and overcome the stressful conditions. Thus, ELF MF treatment activated both glycogen and lipid catabolism, while SMF treatment activated glycogen catabolism but lipid synthesis in fat body at the same time. These findings also suggest that

under the stress provoked by the SMF there was a balance between energy production and loss, while in the case of the ELF MF, the priority was energy expenditure leading to the depletion of energy depots, observed here as reduced fat body mass.

In insects, AKHs have a central role in control of energy metabolism and are involved in the mobilization of carbohydrates and lipids for energy-consuming activities (Gäde 2004; Lorenz and Gäde 2009). Biogenic amines, predominantly octopamine, regulate the activity of glycogen phosphorylase, the enzyme involved in glycogen degradation (Van der Horst et al. 2001). Octopamine, frequently named the "stress hormone", was also responsible for rapid mobilization of lipids for locust flight (Orchard et al. 1993) and for increased amounts of lipids and glycogen in *Acheta domesticus* (Fields and Woodring 1991). Finally, the insulin-like neurohormone bombyxin alters carbohydrate metabolism, including utilization of glycogen, enhanced hydrolysis of trehalose in hemolymph and its uptake by tissue cells (Satake et al. 1997; Meyer-Fernandes et al. 2000). Therefore, it could be assumed that the observed effects of the SMF and ELF MF on glycogen and total lipid content were achieved indirectly by an influence on neurohormonal factors involved in the regulation of energy metabolism in insects. This scenario seems acceptable given the already demonstrated impact of an MF on the activity of neurosecretory neurons in gypsy moth caterpillars and yellow mealworm pupae, as well as octopamine concentrations in cockroaches (Wyszkowska et al. 2006; Perić-Mataruga et al. 2008; Ilijin et al. 2011).

It is known that AKHs and octopamine also have very important roles in the regulation of insect locomotion (Socha et al. 1999; Pflüger and Duch 2000). Thus, AKHs stimulate locomotion (Socha et al. 1999) and mobilize various substrates (lipids, carbohydrates, proline) from stores in insect fat bodies during locomotion and flight (Gäde and Auerswald 2003; Lorenz

et al. 2004; Gálíková et al. 2015). Mobilized substrates undergo oxidation in the muscles, which is predominantly based on carbohydrate oxidation in cockroaches (Gäde and Auerswald 2003). Octopamine plays a major neuromodulatory role in regulating various insect behaviors including locomotion, flight muscle activity and lipid and carbohydrate mobilization. This prepares insects for periods of extended activity or assists recovery from times of increased energy demand (Pflüger and Duch 2000; Farooqui 2012). In the present study chronic exposure of *B. dubia* nymphs to either SMF or ELF MF for 5 months increased all examined parameters of locomotion (travel distance, time in movement, average speed while in motion). These findings are consistent with the already observed influence of MF on locomotion of other insect species (Todorović et al. 2013; Dimitrijević et al. 2014; Pavlović et al. 2016; Wyszowska et al. 2016; Zmejkoski et al. 2017) and could be attributed to effects on AKHs and octopamine, as key factors in the control of insect locomotion.

In light of our recently published finding, an explanation for the observed changes in locomotion should be also sought in the effects of SMF and ELF MF on cholinergic transmission in the brain of *B. dubia* nymphs (Ilijin et al. 2020). In insects, acetylcholine (ACh) is a neurotransmitter involved in the control of locomotion and acetylcholinesterase (AChE) is an enzyme that catalyzes its hydrolysis (Osborne 1996). The amount of synthesized and released neurotransmitters depends on the intracellular Ca^{2+} concentration (Augustine 2001), while the activity of neurons depends on the change in the function of voltage-dependent Na^+ channels (Marchionni et al. 2006; Aldinucci et al. 2009; Cuccurazzu et al. 2011). It is known that MF may increase the membrane potential and voltage sensors move up towards the extracellular matrix (Gapeyev et al. 2001; Bauréus et al. 2013; He et al. 2013; Pall 2013; Lu et al. 2015), resulting in an increased number of open-voltage-dependent Ca^{2+} and Na^+ channels, and consequently

altered neuronal activity and the amount of neurotransmitters released. On the other hand, the reactive oxygen species (ROS) generated after exposure to MFs change the cell membrane properties, e.g. their current and potential, ionic gradients and level of ion channel openness (Kourie 1998; Annunziato et al. 2002). As we have already shown, the activity of the enzyme of antioxidative defense in the gut of *B. dubia* nymphs increased after long-term exposure to both SMF (110 mT) and ELF MF (50 Hz, 10 mT) (Todorović et al. 2019). It can be assumed that the same scenario is in the brain of *B. dubia* nymphs, namely that the applied MFs generate ROS that induce changes in membrane properties and amount of Ca^{2+} ions in neurons, including cholinergic. In addition, long-term exposure to both MFs had a direct effect on cholinergic transmission in the brain of *B. dubia* nymphs causing a decrease in the activity of AChE (Ilijin et al., 2020) and thus excessive ACh accumulation in the synaptic cleft. Having in mind these findings, it is possible that changes in this neurotransmitter determine the behavioral patterns of *B. dubia* nymphs.

Finally, the question remains as to the consequences of such treatment in other organisms and humans. By affecting metabolism, applied MFs could interfere with other energy demanding processes (not only locomotion) in animals. In addition, changes in locomotion could be reflected in performing daily activities involving the movement of animals. For example, it is known that some direct physiological effects on ion channels could well produce immediate behavioral responses and underpin avoidance movements to high levels of ELF electromagnetic field around overhead power lines which could then act as barriers to animals' their movement (Wyszkowska et al. 2016). In humans living and working in the presence of these MFs, prolonged exposure could have adverse health effects, especially in individuals with metabolic and motor disorders. It is very important to point out here the possible difference in sensitivity of

cockroaches and humans to the applied MFs. As already shown for radiation, cockroaches are more resistant than humans (Berenbaum 2001) and thus it is quite expected that the observed effects of MFs in cockroaches are more pronounced in humans.

Conclusion

These findings indicate that chronic exposure to the applied MFs could be considered as a stress affecting different aspects of nymph functioning. Particularly interesting is that *B. dubia* nymphs possess different strategies for energy use in response to the SMF and ELF MF in order to satisfy the increased demands for fuel needed for locomotion and other activities under stressful conditions.

Funding information

This study was supported by the Serbian Ministry of Education, Science and Technological Development, Contract 451-03-68/2020-14/200007.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

- Alamer AH. 2013. Endocrine control of fat body composition and effects of the insect growth regulators methoprene and pyriproxyfen on the development and reproduction of the Argentinian cockroach, *Blaptica dubia* Serville (Blattaria: Blaberidae). PhD Dissertation. Faculty of Biology, Chemistry & Earth Sciences, The University of Bayreuth, Germany.
- Aldinucci C, Carretta A, Maiorca SM, Leoncini S, Signorini C, Ciccoli L, Pessina GP. 2009. Effects of 50 Hz electromagnetic fields on rat cortical synaptosomes. *Toxicol Ind Health* 25(4-5):249-252.
- Annunziato L, Pannaccione A, Cataldi M, Secondo A, Castaldo P, Di Renzo G, Tagliatela M. 2002. Modulation of ion channels by reactive oxygen and nitrogen species: a pathophysiological role in brain aging? *Neurobiol Aging* 23(5):819-834.
- Arrese EL, Soulages JL. 2010. Insect fat body: energy, metabolism, and regulation. *Annu Rev Entomol.* 55:207-225.
- Augustine GJ. 2001. How does calcium trigger neurotransmitter release? *Curr Opin Neurobiol.* 11:320-326.
- Banks AN, Srygley RB. 2003. Orientation by magnetic field in leaf-cutter ants, *Atta colombica* (Hymenoptera: Formicidae). *Ethology.* 109(10):835-846.
- Bauréus Koch CL, Sommarin M, Persson BR, Salford LG, Eberhardt JL. 2003. Interaction between weak low frequency magnetic fields and cell membranes. *Bioelectromagnetics* 24(6):395-402.
- Beenackers AM, Van der Horst DJ, Van Marrewijk WJ. 1985. Insect lipids and lipoproteins, and their role in physiological processes. *Prog Lipid Res.* 24(1):19-67.
- Berenbaum M. 2001. Rad Roaches. *Am Entomol.* 47(3):132-133.

Borgmann A, Büschges A. 2015. Insect motor control: methodological advances, descending control and inter-leg coordination on the move. *Curr Opin Neurobiol.* 33:8-15.

Chino H. 1997. Physiological significance of lipid transport by lipophorin for long-distance flight in insects. *Comp Biochem Physiol B Biochem Mol Biol.* 117(4):455-461.

Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C. 2011. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Exp Neurol.* 226(1):173-182.

Dimitrijević D, Savić T, Anđelković M, Prolić Z, Janać B. 2014. Extremely low frequency magnetic field (50 Hz, 0.5 mT) modifies fitness components and locomotor activity of *Drosophila subobscura*. *Int J Radiat Biol.* 90(5):337-343.

Djawdan M, Chippindale AK, Rose MR, Bradley TJ. 1998. Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol Zool.* 71(5):584-594.

Elferchichi M, Mercier J, Coisy-Quivy M, Metz L, Lajoix AD, Gross R, Belguith H, Abdelmelek H, Sakly M, Lambert K. 2010. Effects of exposure to a 128-mT static magnetic field on glucose and lipid metabolism in serum and skeletal muscle of rats. *Arch Med Res.* 41(5):309-314.

Farooqui T. 2012. Review of octopamine in insect nervous system. *Open Access Insect Physiol.* 4:1.

Fields PE, Woodring JP. 1991. Octopamine mobilization of lipids and carbohydrates in the house cricket, *Acheta domesticus*. *J Insect Physiol.* 37(3):193-199.

Gäde G. 2004. Regulation of intermediary metabolism and water balance of insects by neuropeptides. *Annu Rev Entomol.* 49:93-113.

Gäde G, Auerswald L. 2003. Mode of action of neuropeptides from the adipokinetic hormone family. *Gen Comp Endocrinol.* 132(1):10-20.

Gáliková M, Diesner M, Klepsatel P, Hehlert P, Xu Y, Bickmeyer I, Predel R, Kühnlein RP. 2015. Energy homeostasis control in *Drosophila* adipokinetic hormone mutants. *Genetics*. 201(2):665-683.

Gapeyev AB, Sokolov PA, Chemeris NK. (2001). Response of membrane-associated calcium signaling systems of the cell to extremely low-frequency external signals with different waveform parameters. *Electromagn Biol Med*. 20(1):107-122.

Ghodbane S, Lahbib A, Sakly M, Abdelmelek H. 2013. Bioeffects of static magnetic fields: oxidative stress, genotoxic effects, and cancer studies. *Biomed Res Int*. 2013:602987.

Guerra PA, Gegear RJ, Reppert SM. 2014. A magnetic compass aids monarch butterfly migration. *Nat Commun*. 5:4164.

Hansen LT, Berthelsen H. 2000. The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). *Appl Anim Behav Sci*. 68(2):163-178.

He YL, Liu DD, Fang YJ, Zhan XQ, Yao JJ, Mei YA. 2013. Exposure to extremely low-frequency electromagnetic fields modulates Na⁺ currents in rat cerebellar granule cells through increase of AA/PGE₂ and EP receptor-mediated cAMP/PKA pathway. *PLoS One* 8(1):e54376.

Hill RW, Wyse GA, Anderson M. 2012. *Animal physiology*. Sunderland, MA: Sinauer Associates.

Hintze-Podufal C, Nierling U. 1986. Der Einßuss der Nahrung auf Entwicklung, Wachstum und Prareproduktionsphase von *Blaptica dubia* Stal (Blaberoidea, Blaberidae). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*. 59: 177-186.

Hughes S, El Haj AJ, Dobson J, Martinac B. 2005. The influence of static magnetic fields on mechanosensitive ion channel activity in artificial liposomes. *Eur Biophys J*. 34(5):461-468.

Ilijin L, Vlahović M, Mrdaković M, Mirčić D, Prolić Z, Lazarević J, Perić-Mataruga V. 2011. The effects of acute exposure to magnetic fields on morphometric characteristics of bombyxin-producing neurosecretory neurons in gypsy moth caterpillars. *Int J Radiat Biol.* 87(5):461-471.

Ilijin L, Mrdaković M, Todorović D, Vlahović M, Grčić A, Filipović A, Perić-Mataruga V. 2020. Biological effects of chronic exposure of *Blaptica dubia* (Blattodea: Blaberidae) nymphs to static and extremely low frequency magnetic fields. *An Acad Bras Ciênc.*, in press.

Jankowska M, Pawlowska-Mainville A, Stankiewicz M, Rogalska J, Wyszowska J. 2015. Exposure to 50 Hz electromagnetic field changes the efficiency of the scorpion alpha toxin. *J Venom Anim Toxins Incl Trop Dis.* 21:38.

Klotz J, Jander R. 2003. Magnetic sense. In: Resh V, Cardé R, editors. *Encyclopedia of insects.* 1st ed. London: Academic Press; p. 670-672.

Kooijman S. 2000. *Dynamic energy and mass budgets in biological systems.* Cambridge, UK: Cambridge University Press.

Kourie JJ. 1998. Interaction of reactive oxygen species with ion transport mechanisms. *Am J Physiol.* 275(1):C1-C24.

Kumosani TA, Qari MH. 2003. The effect of magnetic field on the biochemical parameters of mice blood. *Pak J Med Sci.* 19(1):36-40.

Leszczynski D. 2005. Rapporteur report: Cellular, animal and epidemiological studies of the effects of static magnetic fields relevant to human health. *Prog Biophys Mol Bio* 87: 247-253.

Levitt BB, Lai H. 2010. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. *Environ Rev.* 18:369-395.

Lorenz MW, Gäde G. 2009. Hormonal regulation of energy metabolism in insects as a driving force for performance. *Integr Comp Biol.* 49(4):380-392.

Lorenz MW, Zemek R, Kodrík D, Socha R. 2004. Lipid mobilization and locomotor stimulation in *Gryllus bimaculatus* by topically applied adipokinetic hormone. *Physiol Entomol.* 29:146-151.

Lu XW, Du L, Kou L, Song N, Zhang YJ, Wu MK, Shen JF. 2015. Effects of moderate static magnetic fields on the voltage-gated sodium and calcium channel currents in trigeminal ganglion neurons. *Electromagn Biol Med.* 34(4):285-292.

Maliszewska J, Marciniak P, Kletkiewicz H, Wyszowska J, Nowakowska A, Rogalska J. 2018. Electromagnetic field exposure (50 Hz) impairs response to noxious heat in American cockroach. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol.* 204(6):605-611.

Marchionni I, Paffi A, Pellegrino M, Liberti M, Apollonio F, Abeti R, Fontana F, D'Inzeo G, Mazzanti M. 2006. Comparison between low-level 50 Hz and 900 MHz electromagnetic stimulation on single channel ionic currents and on firing frequency in dorsal root ganglion isolated neurons. *Biochim Biophys Acta* 1758(5):597-605.

Martin JR. 2003. Locomotor activity: a complex behavioural trait to unravel. *Behav Processes.* 64(2):145-160.

Martin JP, Guo P, Mu L, Harley CM, Ritzmann RE. 2015. Central-complex control of movement in the freely walking cockroach. *Curr Biol.* 25(21):2795-2803.

Meyer-Fernandes JR, Gondim KC, Wells MA. 2000. Developmental changes in the response of larval *Manduca sexta* fat body glycogen phosphorylase to starvation, stress and octopamine. *Insect Biochem Mol Biol.* 30(5):415-422.

Nässel DR, Winther AM. 2010. *Drosophila* neuropeptides in regulation of physiology and behavior. *Prog Neurobiol.* 92(1):42-104.

Oguri E, Steele JE. 2003. A novel function of cockroach (*Periplaneta americana*) hypertrehalosemic hormone: translocation of lipid from hemolymph to fat body. *Gen Comp Endocrinol.* 132(1):46-54.

Orchard I, Ramirez JM, Lange AB. 1993. A multifunctional role for octopamine in locust flight. *Ann Rev Entomol.* 38(1):227-249.

Osborne RH. 1996. Insect neurotransmission: Neurotransmitters and their receptors. *Pharmacol Ther.* 69(2):117-142.

Pall ML. 2013. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. *J Cell Mol Med.* 17(8):958-965.

Pavlović D, Petković B, Čurčić S, Todorović D, Vesović N, Pantelić D, Perić-Mataruga V. 2016. Increased motor activity of the beetle *Laemostenus punctatus* caused by a static magnetic field of 110 mT. *Entomol Exp Appl.* 160(2):188-194.

Perić-Mataruga V, Prolić Z, Nenadović V, Vlahović M, Mrdaković M. 2008. The effect of a static magnetic field on the morphometric characteristics of neurosecretory neurons and corpora allata in the pupae of yellow mealworm *Tenebrio molitor* (Tenebrionidae). *Int J Radiat Biol.* 84(2):91-98.

Pflüger HJ, Duch C. 2000. The functional role of octopaminergic neurons in insect motor behavior. *Acta Biol Hung.* 51(2-4):343-348.

Rall BC, Vucic-Pestic O, Ehnes RB, Emmerson M, Brose U. 2010. Temperature, predator-prey interaction strength and population stability. *Glob Change Biol.* 16(8):2145-2157.

Rauš S, Todorović D, Prolić Z. 2009. Viability of old house borer (*Hylotrupes bajulus*) larvae exposed to a constant magnetic field of 98 mT under laboratory conditions. *Arch Biol Sci.* 61(1):129-134.

Riveros AJ, Srygley RB. 2010. Migration, orientation and navigation: magnetic compasses in insects. In: Breed MD, Moore J, editors. Encyclopedia of animal behavior. Oxford: Elsevier/Academic Press; p. 305-313.

Roeder T. 1999. Octopamine in invertebrates. *Prog Neurobiol.* 59(5):533-561.

Satake S, Masumura M, Ishizaki H, Nagata K, Kataoka H, Suzuki A, Mizoguchi A. 1997. Bombyxin, an insulin-related peptide of insects, reduces the major storage carbohydrates in the silkworm *Bombyx mori*. *Comp Biochem Physiol B Biochem Mol Biol.* 118(2):349-357.

Savić T, Janać B, Todorović D, Prolić Z. 2011. The embryonic and post-embryonic development in two *Drosophila* species exposed to the static magnetic field of 60 mT. *Electromagn Biol Med.* 30(2):108-114.

Serway CN, Kaufman RR, Strauss R, de Belle JS. 2009. Mushroom bodies enhance initial motor activity in *Drosophila*. *J Neurogenet.* 23(1-2):173-184.

Sheppard DM, Li J, Henbest KB, Neil SR, Maeda K, Storey J, Schleicher E, Biskup T, Rodriguez R, Weber S et al. 2017. Millitesla magnetic field effects on the photocycle of an animal cryptochrome. *Sci Rep.* 7:42228.

Socha R, Kodrík D, Zemek R. 1999. Adipokinetic hormone stimulates insect locomotor activity. *Naturwissenschaften.* 88:85-86.

Stanojević V, Prolić Z, Savić T, Todorović D, Janać B. 2005. Effects of extremely low frequency (50 Hz) magnetic field on development dynamics of the housefly (*Musca domestica* L.). *Electromagn Biol Med.* 24(2):99-107.

Starick NT, Longstaff BC, Condon B. 2005. The influence of fluctuating low-level magnetic fields on the fecundity and behaviour of *Rhyzopertha dominica* (F.). *J Stored Prod Res.* 41(3):255-270.

Steele JE. 1999. Activation of fat body in *Periplaneta americana* (Blattoptera: Blattidae) by hypertrehalosemic hormones (HTH): New insights into the mechanism of cell signalling [journal article]. *Eur J Entomol.* 96(3):317-322.

Stone JV, Mordue W. 1980. Adipokinetic hormone. In: Miller TA, editor. Neurohormonal techniques in insects. New York: Springer; p. 31-80.

Todorović D, Ilijin L, Mrdaković M, Vlahović M, Filipović A, Grčić A, Perić-Mataruga V. 2019. Long-term exposure of cockroach *Blattella germanica* (Insecta: Blattellidae) nymphs to magnetic fields of different characteristics: effects on antioxidant biomarkers and nymphal gut mass. *Int J Radiat Biol.* 95(8):1185-1193.

Todorović D, Marković T, Prolić Z, Mihajlović S, Rauš S, Nikolić L, Janać B. 2013. The influence of static magnetic field (50 mT) on development and motor behaviour of *Tenebrio molitor* (Insecta, Coleoptera). *Int J Radiat Biol.* 89(1):44-50.

Todorović D, Mirčić D, Ilijin L, Mrdaković M, Vlahović M, Prolić Z, Perić-Mataruga V. 2012. Effect of magnetic fields on antioxidative defense and fitness-related traits of *Baculum extradentatum* (insecta, phasmatodea). *Bioelectromagnetics.* 33(3):265-273.

Van der Horst DJ, Van Marrewijk WJ, Diederer JH. 2001. Adipokinetic hormones of insect: release, signal transduction, and responses. *Int Rev Cytol.* 211:179-240.

WHO. 2006. Static Fields. Environmental Health Criteria 232. Geneva, Switzerland: World Health Organization.

WHO. 2007. Extremely Low Frequency Fields. Environmental Health Criteria 238. Geneva, Switzerland: World Health Organization.

Wu H. 2013. Biology of *Blattella germanica* (Blattodea: Blattellidae). PhD thesis. Graduate Faculty of Auburn University.

- Wyatt GR, Kale GF. 1957. The chemistry of insect hemolymph. II. Trehalose and other carbohydrates. *J Gen Physiol.* 40(6):833-847.
- Wyszkowska J, Shepherd S, Sharkh S, Jackson CW, Newland PL. 2016. Exposure to extremely low frequency electromagnetic fields alters the behaviour, physiology and stress protein levels of desert locusts. *Sci Rep.* 6:36413.
- Wyszkowska J, Stankiewicz M, Krawczyk A, Zyss T. 2006. Octopamine activity as indicator of electromagnetic field influence on insect nervous system. Ohrid, Macedonia: SAEM–First Macedonian-Polish symposium on applied electromagnetics. p. 83-84.
- Xu J, Pan W, Zhang Y, Li Y, Wan G, Chen F, Sword GA, Pan W. 2017. Behavioral evidence for a magnetic sense in the oriental armyworm, *Mythimna separata*. *Biol Open.* 6(3):340-347.
- Yang Y, Yan Y, Zou X, Zhang C, Zhang H, Xu Y, Wang X, Janos P, Yang Z, Gu H. 2011. Static magnetic field modulates rhythmic activities of a cluster of large local interneurons in *Drosophila* antennal lobe. *J Neurophysiol.* 106(5):2127-2135.
- Zannella S. 1997. Biological effects of magnetic fields. CAS - CERN Accelerator School: Measurement and Alignment of Accelerator and Detector Magnets; 11 - 17 Apr 1997; Anacapri, Italy. p. 375-386 (CERN-1998-1005).
- Zmejkoski D, Petković B, Pavković-Lučić S, Prolić Z, Anđelković M, Savić T. 2017. Different responses of *Drosophila subobscura* isofemale lines to extremely low frequency magnetic field (50 Hz, 0.5 mT): fitness components and locomotor activity. *Int J Radiat Biol.* 93(5):544-552.

Figure legends

Figure 1. The influence of chronic exposure to a static magnetic field (SMF, 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) for 5 months on glycogen content (A) and total lipid content (B) in the fat body of *B. dubia* nymphs. Results are presented as means \pm S.E.M. (n = 10 nymphs per group). Different letters (a, b, c) indicate significant differences between groups (LSD test).

Figure 2. The influence of chronic exposure to a static magnetic field (SMF, 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) for 5 months on locomotion, expressed as travel distance (A), time in movement (B) and average speed while in motion (C), of *B. dubia* nymphs. Results are presented as means \pm S.E.M. (n = 10 nymphs per group) during a 10-min period. Different letters (a, b) indicate significant differences between groups (LSD test).

Table 1. The influence of chronic exposure to a static magnetic field (SMF, 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) for 5 months on body mass and fat body mass of *B. dubia* nymphs. Results are presented as means \pm S.E.M. (n = 10 nymphs per group). Different letters (a, b) indicate significant differences between groups (LSD test).

	Nymph body mass (mg)		Fat body mass (mg)
	after 1 month	after 5 months	after 5 months
Control	43.0 \pm 3.9 ^a	663.9 \pm 100.6 ^a	32.4 \pm 1.1 ^a
SMF	40.9 \pm 1.6 ^a	275.5 \pm 33.0 ^b	29.1 \pm 1.9 ^a
ELF MF	45.1 \pm 3.1 ^a	351.7 \pm 47.5 ^b	24.3 \pm 1.0 ^b

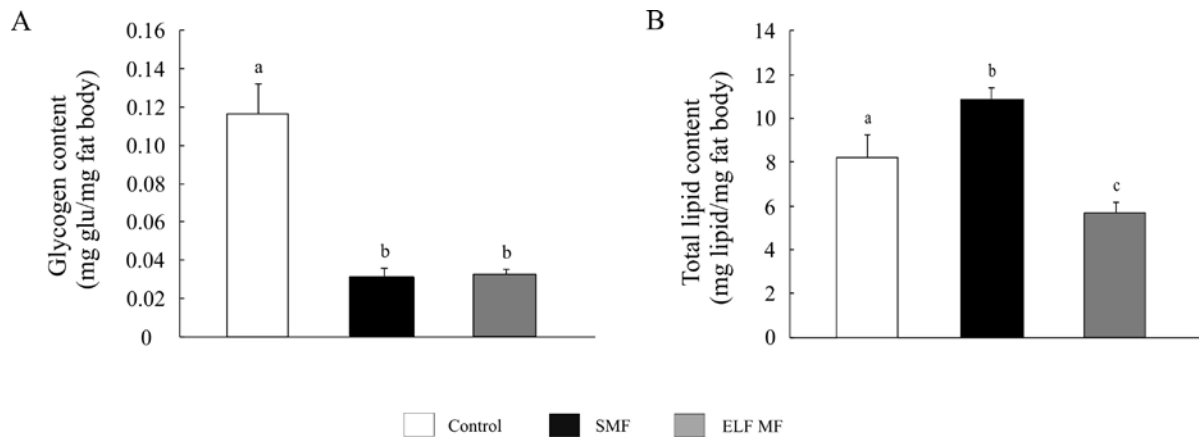


Figure 1. The influence of chronic exposure to a static magnetic field (SMF, 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) for 5 months on glycogen content (A) and total lipid content (B) in the fat body of *B. dubia* nymphs. Results are presented as means \pm S.E.M. (n = 10 nymphs per group). Different letters (a, b, c) indicate significant differences between groups (LSD test).

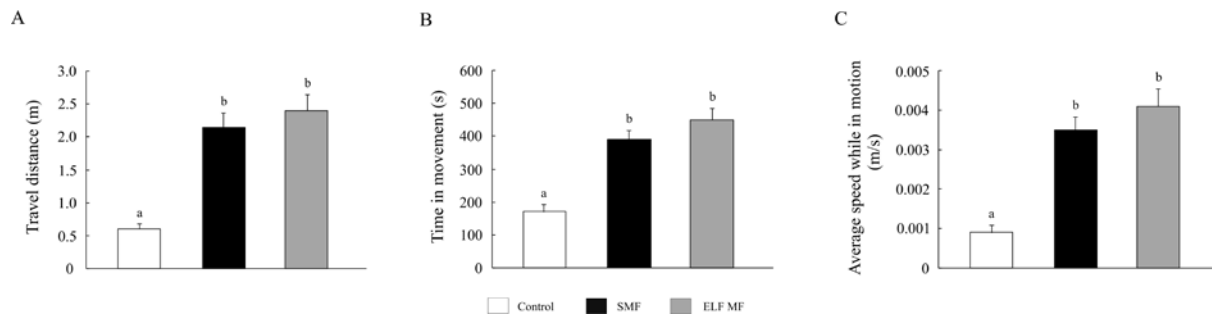


Figure 2. The influence of chronic exposure to a static magnetic field (SMF, 110 mT) and an extremely low frequency magnetic field (ELF MF; 50 Hz, 10 mT) for 5 months on locomotion, expressed as travel distance (A), time in movement (B) and average speed while in motion (C), of *B. dubia* nymphs. Results are presented as means \pm S.E.M. (n = 10 nymphs per group) during a 10-min period. Different letters (a, b) indicate significant differences between groups (LSD test).