SOME PECULIARITIES OF RAT'S BEHAVIOUR AT THE ACTION OF HIGH TEMPERATURES

VRABIE Valeria, CIOCHINĂ Valentina, BODORIN Cornelia

Abstract. Understanding animal responses to the action of environmental stressors is of increasing importance under changing climate regimes. Extreme ambient temperature is one of the main abiotic factors which directly influence the viability of the species, causing significant losses of biodiversity. Animals are forced to adapt, especially by changing their behaviour, as the action of stressors increases. In order to highlight some peculiarities of the animals' behaviour in conditions of high ambient temperatures, in laboratory experiments, on white laboratory rats, Wistar line, the action of high temperature (+40 °C) was studied on their locomotor activity, orientation reaction and emotional state (anxiety), depending on the circadian rhythm of animal activity. It has been established that the thermal factor, namely the high temperature, significantly affects the behaviour of laboratory animals, inhibiting the orientation reaction, locomotion, grooming and inducing a high level of anxiety in nocturnal rats, which can be considered as a reaction of accommodation to the stress factor. The change in the circadian rhythm, namely the morning feeding of the rats (morning group) conditioned the increase of the orientation reaction and the locomotor activity, and the decrease of anxiety state in stressed animals. Thus, due to the high temperatures, and depending on the circadian rhythm, the behaviour of rats is quite complex and oriented towards adapting as quickly as possible to the new environmental conditions.

Keywords: rats, higher temperature, stress, behaviour, anxiety.

Rezumat. Unele particularități ale comportamentului șobolanilor la acțiunea temperaturii înalte. Cunoașterea modului în care animalele răspund la acțiunea factorilor de stres ai mediului ambiant prezintă o importanță tot mai mare în condițiile schimbării regimurilor climatice. Temperatura extremă a mediului ambiant este unul din factorii abiotici principali, care influențează direct viabilitatea speciilor, cauzând pierderi semnificative ale biodiversității. Animalele sunt forțate să se adapteze, în special modificându-și comportamentul, pe măsură ce acțiunea factorilor nefavorabili se amplifică. Pentru a evidenția unele particularități ale comportamentului animalelor în condiții de temperaturi ridicate ale mediului ambiant, în experimente de laborator, pe șobolani albi de laborator, linia Wistar, a fost studiată acțiunea temperaturii ridicate (+40 °C) asupra activității locomotorii, reacției de orientare și stării emoționale (anxietății) a acestora, în dependență de ritmul circadian de activitate a animalelor. A fost stabilit că factorul termic, și anume temperatura înaltă afectează semnificativ comportamentul animalelor de laborator, inhibând reacția de orientare, locomoția, timpul de îngrijire și inducând un nivel înalt de anxietate la șobolanii din grupa nocturnă, ceea ce poate fi considerată ca o reacție de acomodare la factorul stres. Modificarea ritmului circadian, și anume hrănirea dimineața a șobolanilor (grupa matinală), a condiționat majorarea reacției de orientare și a activității locomotorii și micșorarea stării de anxietate la animalele stresate. Astfel, la acțiunea temperaturilor înalte și în funcție de ritmul circadian, comportamentul șobolanilor este destul de complex și orientat spre acomodarea cât mai rapidă la noile condiții de mediu

Cuvinte cheie: șobolani, temperatura înaltă, stres, comportament, anxietate.

INTRODUCTION

Climate change has a significant impact on living organisms, favouring or preventing the survival and reproduction of various species of organisms. Of the climatic elements, the main abiotic factor is temperature. It has been established that "*the ~ 1ºC rise in mean global temperature is causing serious and often unexpected impacts on species, affecting their abundance, genetic composition, behaviuor and survival*" (NASA's Goddard Institute for Space Studies, 2022).

The response of different species of animals and plants to changes in the environment (as a result of climate change) can desynchronize ecological interactions and threaten the function of the ecosystem. That is, different species of organisms have different climatic sensitivities, especially when the temperature changes. As the ambient temperature increases, changes in metabolic processes occur, which affect the physiology and behaviourbehaviour of living things, even at the ecosystem level (BROWN et al., 2004; ANGILLETTA, 2009).

Of course, the dependence on physiological processes and thermal rate behaviourbehaviour is the most predictable for ectothermic organisms, which, in contrast to endotherms, do not typically maintain a constant body temperature through homeostatic processes. Both endotherms and ectotherms have adaptations – features that arose by natural selection – that help them maintain a healthy body temperature. These adaptations can be behaviourbehavioural, anatomical, or physiological (WILLIAMS et al., 2008; HUEY et al., 2012).

The mammal species develop different physiological and behaviourbehavioural strategies to cope with both decreased and increased levels of ambient temperature. Thermoregulation is a key feature in the maintenance of homeostasis and behavioural adjustments are available to lower the autonomic work, and thus reduce the energy costs of thermoregulatory responses (TERRIEN et al., 2011). Changes in animal behaviour in response to temperature changes are a feature that determines the plasticity of species adaptation to new environmental conditions (REFINETTI, 2010; KJAERSGAARD et al., 2015). Behavioural adjustment of living organisms, especially animals, are considered an immediate compensatory reaction, a buffer with which animals avoid the immediate negative effects of environmental variation (MILLING et al., 2017; RADCHUK et al., 2019; GUNN et al., 2021). The activity patterns in many endotherms change seasonally. Endothermic animals (birds and mammals) reduce activity levels at extreme temperature variation (VICKERY & BIDER, 1981; STAWSKI & GEISER, 2020). By changing their behaviour, animals can also gain more information on the altered environment.

In a study of the Climate Sensitivity Profile applied to 10,003 terrestrial and aquatic phenological data sets, it was established that the magnitude and timing of climate sensitivity varied markedly among organisms within taxonomic and trophic groups. Secondary consumers showed consistently lower climate sensitivity than other groups. The authors assumed that species lower down on the food chain will change their behaviour more as temperatures continue to rise (THACKERAY et al., 2016). In this case, hunting and foraging patterns are changing, as food resources become sparser. Animals are forced to adapt to the new conditions, in which they often have to go closer to human settlements for foraging.

The ambient temperature is capable of affecting behaviour of animals with different of circadian rhythms. For example, changes in ambient temperature switch the activity patterns of nocturnal mice to diurnal patterns (van der VINNE et al., 2014) and of diurnal rodents to nocturnal patterns (VIVANCO et al., 2010). Generally, the rodents have been shown to exhibit a great deal of plasticity to temperature fluctuations (FOGO et al., 2019). However, for most rodents, it is difficult to maintain a normal body temperature due to their small size. The regulation is mainly due to the decrease or increase of internal heat production (REPPERT & WEAVER, 2002).

Thus, with the rapid rise in global temperature, research has been conducted to assess the consequences of this change on wildlife. The impact of the thermal factor on the phenology, distribution and demography of wildlife populations has been well studied. Less documented are the peculiarities of the behaviour of animals related to their cognitive ability, which allows survival in extreme conditions. Organisms use external temperature as an indication of the environment, useful for training biological and behavioural rhythms (FULLER et al., 2014).

Laboratory studies on the behavioural and cognitive response of animals to changes in abiotic factors may not accurately predict how animals respond to their natural habitats, but they may reveal valuable information about patterns of behaviour in environmental change.

The aim of the study was to study the influence of temperature on the behaviour of rats kept in relatively comfortable conditions depending on the circadian rhythm of animal activity.

MATERIAL AND METHODS

The object of study was white laboratory rats, males, one month old, weight 173-226 g, maintained 12 in 4 cells with dimensions 40 x 50 x 20 cm in artificial light and temperature between: +10,5 °C - + 20 °C. The laboratory rats were divided into 2 groups: the "morning" group (which fed during the day from 7^{30} to 19^{30}) and the "night" group (which fed during the night, in the absence of light from 19^{30} until at 7^{30}). The investigation of the behaviour of two groups of animals – nocturnal and morning – was carried out on the grounds that any change in the natural environment conditions, including temperature, could change the period of activity of the animals and the hunting and foraging time.

In order to study the influence of temperature factor on behaviour, these 2 groups were divided into 2 subgroups of 6 animals each: control animals and rats subjected to temperature stress. The high temperature conditions were created by placing the animals in the laboratory thermostat type TC-80M, during 10 days, for 15 minutes at a temperature of 40 °C, every day. Changes in rat behaviour over 5 minutes were noted daily after 15 minutes of high temperature stress.

The behavioural study was performed using the "open field" method. The "open field" test was proposed by HALL (1936) for recording animal behaviour in response to potentially dangerous new stimuli. Testing animals in the open field using the method developed by HALL is one of the most commonly used methods for studying behaviour and mental processes in small laboratory animals. A cylindrical open field device was used for this.

The arena of the device was divided into 19 sections (squares), 12 of which were peripheral and 7 - central. Each rat was individually tested. The duration of the animal testing in th open field was 5 min. During this time, the following parameters were characterized that characterize the behaviour: 1) locomotion (number of intersected squares); 2) orientation reaction (number of vertical lifts); 3) the number of defecations (number of bowls) in the tests and the number of urinations during testing (these two parameters are considered indicators of emotional anxiety; 5) grooming, its duration. At least, 60 minutes before testing in the open field, any handling of animals shall be excluded.

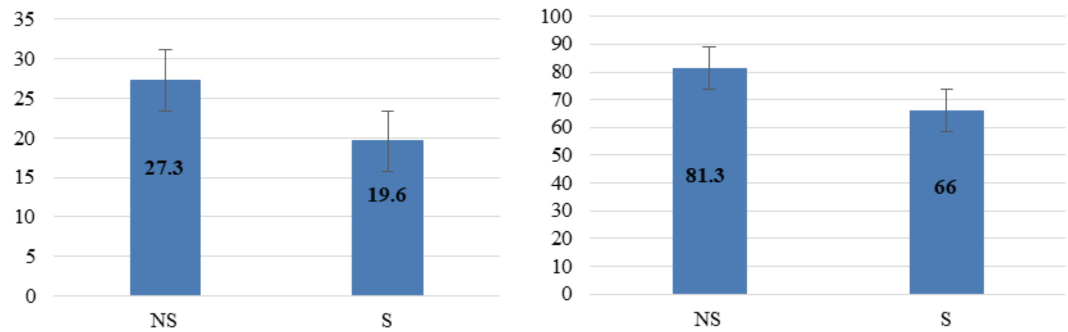
The data obtained were statistically processed using the Student's t-test to determine probability and error.

The experiments were performed in the Vivarium of the Institute of Physiology and Sanocreatology, in accordance with Directive 86/609 / EEC of 24 November 1986 on the Protection of Animals Used for Experimental and Other Scientific Purposes and were approved by the Methodical and Ethics Committees of the Institute of Physiology and Sanocreatology.

RESULTS

It is known that temperature, from an ecological point of view, is a limiting factor that influences the lifestyle of living organisms, the spread and geographical distribution of species, and temperature variations influence the activity of biocenoses in the same geographical areas (LACETERA et al., 2019). For these reasons, a proposal was made to study the influence of high temperature on the behaviour of laboratory animals (white rats, Wistar line, males). Behaviour in rats was studied in two groups of animals: fed in the morning ("morning" group) and fed in the evening ("night" group), according to the "open field" method.

According to the obtained data, it was established that the thermal factor, i.e., the high temperature, significantly affects the behaviour of laboratory animals (Fig. 1).

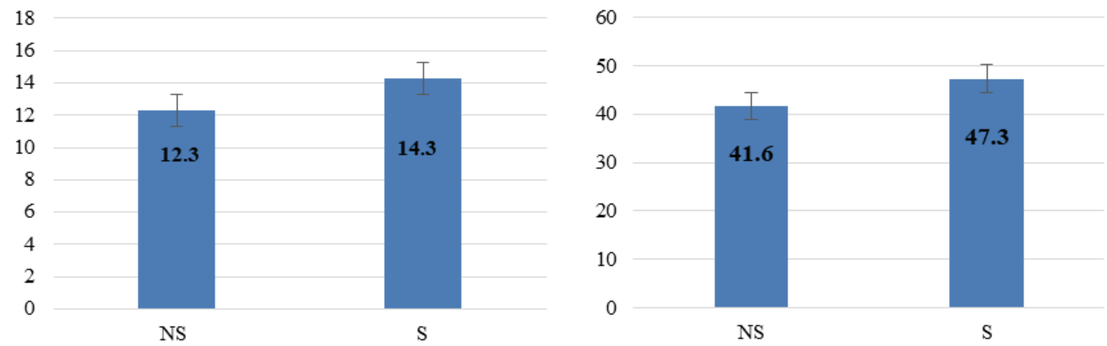


A B

Figure 1. Orientation reaction (number of vertical lifts) (A) and locomotion (number of intersecting squares) (B) in control (NS) and stressed (S) nocturnal rats.

Thus, as the temperature rises, the number of orientation reactions of stressed rats in the "night" group decreases by about 30% compared to the control (unstressed rats). Also, the nocturnal group found a decrease in locomotion (the number of intersected squares) in the case of exposure of rats to thermal shock, by 18%. Probably, in this case, the stressed animals, through the less active behaviour, try to adapt to the created stress conditions.

The behaviour of the rats in the "morning" group differs from the nocturnal one by an increase in the orientation reaction and the locomotor activity of the stressed animals, compared to the unstressed animals from the control group, respectively by 16% and 13% (Fig. 2). At the same time, the data obtained show that rats in the morning group have less activity compared to rats from the "night" group (Figs. 1 and 2).



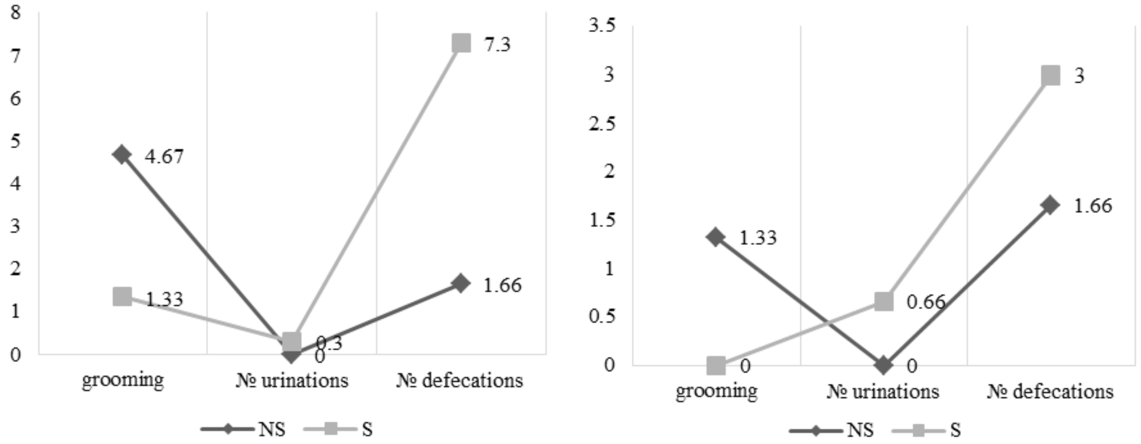
A B

Figure 2. Orientation reaction (number of vertical lifts) (A) and locomotion (number of intersecting squares) (B) in control (NS) and stressed (S) rats in the morning group

This may be due to the fact that feeding rats during the day, which is the inactive period of rodents, causes food ingestion and leads to internal desynchronization, with the possible result of obesity. Thus, in relatively comforting conditions, unstressed animals in the morning group are mostly inactive, and stressful conditions in the morning group, on the contrary, induce a higher physical activity.

Thus, according to the data obtained, it was established that the thermal factor, i.e., the high temperature, significantly affects the behaviour of laboratory animals.

An important parameter that characterizes stressful behaviour is the level of anxiety. The anxiety level of the rats in the control group and the stressed ones was determined by the duration of grooming, the number of defecations and urinations (Fig. 3).



A B

Figure 3. Indices of anxiety level - "grooming", "number of urinations" and "number of defecations" at rats subjected to heat stress in the "Night Group" (A) and "Morning Group" (B)

Due to the high temperature, rats in the nocturnal group show a high level of anxiety, characterized on the one hand by the reduction of washing time (grooming), and on the other hand – the increase in the number of urinations and defecations. Rats in the "morning" group also reacted to the action of stress by increasing anxiety, but the value of the parameters was lower compared to the "night" group (Fig. 3).

Thus, the self-grooming time for rats in the "night" group was reduced by 3.5 times and in rats in the "morning" group by 1.3 units. The number of defecations and urinations, calculated by the number of bowls that take place for 5 minutes of rats in the open field arena, was the same in the unstressed rats (control) of the "night" group and of the "morning" group. The high temperature, as an environmental limiting factor, determined the induction of stress, which caused an increase in the number of defecations and urinations in both groups of animals – 4.4 times in the rats from the "night" group and 1.8 times in the rats from the "morning" group. The number of urinations has not changed significantly.

This difference in the anxious reaction of rats with a different foraging time can be explained by the fact that the rats in the "morning" group were previously subjected to circadian stress (different feeding time). Subsequent exposure to a limiting factor such as temperature and testing in the open field (which in fact is still a stress factor – new place) can cause a lesser reaction to stress. It can be assumed that an emotional adjustment to the stressors' action takes place.

DISCUSSIONS

When analysing the obtained data, it should be taken into account that the testing of animals in the open field is an additional stress factor to the thermal one, to which the rats were previously subjected. At the same time, feeding the animals in the morning is again a stress factor, as feeding during the day is not characteristic of rats, which are nocturnal animals. Thus, the peculiarities of the behaviour of rats in the nocturnal group represent a natural pattern of behaviour, which can reproduce the reaction of animals to the action of high temperatures.

As mentioned above, high temperature inhibits the behaviour of nocturnal rats, which can be considered an accommodation reaction to the stress factor (SHELTON & ALBERTS, 2018). Changing the feeding time, however, induces disorders of the normal behaviour of rats under stress. However, the animals are finding ways to adapt, which will ensure their survival. Thus, the increase in the orientation reaction and locomotor activity in stressed animals in the morning group is a reaction to compensate for the processes of adaptation of animals with excess body mass.

The action of additional stressors alters the emotional behaviour of rats. Usually, under new conditions or under the action of stressors, experimental animals, in particular rats, react by increasing grooming (FERNÁNDEZ-TERUEL & ESTANISLAU, 2016). This increase serves as an indicator of the level of anxiety in stressed animals. However, the increase in grooming has been documented in the unilateral action of a single stress factor (KALUEFF & TUOHIMAA, 2004, VELOSO et al., 2016).

In the performed experiments, the animals were practically stressed twice and even three times (in the case of the rats in the morning group). Probably for this reason, in rats in the control group (control), compared to stressed animals, the self-grooming time increases. We can assume that the high temperature inhibits the activity of the animals, which was established by the data shown in figures 1 and 2, reducing the time spent self-grooming. On the other hand, the higher number of defecations and urinations in stressed rats in both the night and morning groups indicates a high level of anxiety.

CONCLUSIONS

According to the obtained data, we can conclude that the behaviour of rats to change environmental factors is quite complex, and aimed at adapting as quickly as possible to the new environmental conditions.

Thus, it was determined that high temperature inhibited the behaviour activity of nocturnal rats by reducing locomotion activity, orientation reaction and grooming, leading to a high level of anxiety.

Changing the feeding time of rats induces changes in the (usual) behaviour of rats under stress. Rats in the "morning" group responded to high temperature stress through increase of orientation and locomotion activity, as well as a lower level of anxiety compared to rats in the "night" group.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology in the frame Project 15.817.04.01F.

REFERENCES

ANGILLETTA M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford, UK.

BROWN J. H., GILLOOLY J. F., ALLEN A. P., SAVAGE V. M., WEST G. B. 2004. Toward a metabolic theory of ecology. *Ecology*. Ecological Society of America, USA. **85**: 1771-1789.

FERNÁNDEZ-TERUEL A. & ESTANISLAU C. 2016. Meanings of self-grooming depend on an inverted U-shaped function with aversiveness. *Nature Reviews Neuroscience.* Nature Publishing Group. USA. **17**: 591 https://doi.org/10.1038/nrn.2016.102 (Accessed: April 2, 2022).

FOGO G. M., GOODWIN A. M., KHACHERIAN O. S., LEDBETTER B. J., GALL A. J. 2019. The effects of ambient temperature and lighting intensity on wheel-running behavior in a diurnal rodent, the Nile grass rat (Arvicanthis niloticus). *Journal of Comparative Psychology*. American Psychological Association (APA). USA. **133**(2): 215-222. doi: 10.1037/com0000154. (Accessed: April 13, 2022).

FULLER A., HETEM R. S., MALONEY S. K., MITCHELL D. 2014. Adaptation to heat and water shortage in large, arid-zone mammals. Physiology (Bethesda). American Physiological Society. USA. **29**(3): 159-167. doi: 10.1152/physiol.00049. 2013. (Accessed: April 2, 2022).

GUNN R. L., HARTLEY I. R., ALGAR A. C., NIEMELÄ P. T., KEITH S. A. Understanding behavioural responses to human-induced rapid environmental change: a meta-analysis. *Oikos*. Wiley-Blackwell. USA. 2021; doi: 10.1111/oik.08366 (Accessed: April 2, 2022).

HALL C. S. 1936. Emotional behavior in the rat. III. The relationship between emotionality and ambulatory activity. *Journal of Comparative and Physiological Psychology*. American Psychological Association (APA). **22**(3): 345-352.

HUEY R. B., KEARNEY M. R., KROCKENBERGER A., HOLTUM J. A. M., JESS M., WILLIAMS S. E. 2012. Predicting organismal vulnerability to climate warming: roles of behavior, physiology and adaptation. *Philosophical Transactions of the Royal Society. B*. Royal Society, UK. 367: 1665-1679. doi: 10.1098/rstb.2012.0005 (Accessed: April 10, 2022).

KALUEFF A. V. & TUOHIMAA P. 2004. Grooming analysis algorithm for neurobehavioural stress research. *Brain Brain research. Brain research protocols*. Elsevier. Netherlands. **13**(3): 151-158. doi: 10.1016/j.brainresprot.2004.04.002.

KJAERSGAARD A., BLANCKENHORN W. U., PERTOLDI C., LOESCHKE V., KAUFMANN C., HALD B., PAGES N., BAHRNDORFF S. 2015. Plasticity in behavioural responses and resistance to temperature stress in *Musca domestica*. *Animal Behaviour*. Elsevier. Netherlands. **99**: 123-130. https://doi.org/10.1016/j.anbehav.2014.11.003 (Accessed: April 4, 2022).

LACETERA N. 2019. Impact of climate change on animal health and welfare. *Animal Frontiers*. Frontiers Media SA, Switzerland. **9**(1): 26-31,https://doi.org/10.1093/af/vfy030) (Accessed: April 10, 2022).

MILLING C. R., RACHLOW J. L., JOHNSON T. R., FORBEY J. S., SHIPLEY L. A. 2017. Seasonal variation in behavioral thermoregulation and predator avoidance in a small mammal. *Behavioral Ecology*. Oxford University Press. UK. **28**(5): 1236-1247 https://doi.org/10.1093/beheco/arx084 (Accessed: April 4, 2022).

NASA's Goddard Institute for Space Studies (GISS). 2022. Global land-ocean temperature index. https://climate.nasa.gov/vital-signs/global-temperature/) (Accessed: April 4, 2022).

RADCHUK V., REED T., TEPLITSKY C. 2019. Adaptive responses of animals to climate change are most likely insufficient. *Nature Communications*. Nature Publishing Group. UK. **10:** 3109 https://doi.org/10.1038/s41467-019-10924-4 (Accessed: April 1, 2022).

REFINETTI R. 2010. Entrainment of circadian rhythm by ambient temperature cycles in mice. *Journal of Biological Rhythms*. SAGE Publishing. USA. **25**(4): 247-256. http://dx.doi.org/10.1177/0748730410372074 (Accessed: April 2, 2022).

REPPERT S. M. & WEAVER D. R. 2002. Coordination of circadian timing in mammals. *Nature*. Springer Nature. London, UK. **12**: 935-941. doi: 10. 1038/ nature00965 (Accessed: April 3, 2022).

SHELTON D. S. & ALBERTS J. R. 2018. Development of behavioral responses to thermal challenges. *Developmental Psychobiology*. John Wiley & Sons, Inc. USA. **60**(1):5-14. doi:10.1002/dev.21588 (Accessed: April 20, 2022).

STAWSKI C., & GEISER F. 2020. Growing up in a changing climate: how temperature affects the development of morphological, behavioral and physiological traits of a marsupial mammal. *Frontiers in Psychology*. Frontiers Media SA, Switzerland. https://doi.org/10.3389/fphys.2020.00049 (Accessed: April 8, 2022).

TERRIEN J., PERRET M., AUJARD F. 2011. Behavioral thermoregulation in mammals: a review. *Front Biosci*. Frontiers in Bioscience (Singapore). **16**(4): 1428-1444. doi: 10.2741/3797. (Accessed: April 1, 2022).

THACKERAY S. J., HENRYS P. A., HEMMING D., BELL J. R., BOTHAM M. S., BURTHE S. HELAOUET P. 2016. Phenological sensitivity to climate across taxa and trophic levels. *Nature*. Springer Nature. London, UK. **535** (7611): 241-245 https://doi.org/10.1038/nature18608. (Accessed: April 5, 2022).

VAN DER VINNE V., RIEDE S. J., GORTER J. A., EIJER W. G., SELLIX M. T., MENAKER M. 2014. Cold and hunger induce diurnality in a nocturnal mammal. *Proceedings of the National Academy of Sciences*. United States National Academy of Sciences. **111**: 15256-15260.

VELOSO A. W. N., FILGUEIRAS G. B., LORENZO P., ESTANISLAU C. 2016. Modulation of grooming behavior in rats by different test situations. *Psychology & Neuroscience*. American Physiological Society. USA. **9**(1): 91-104. https://doi.org/10.1037/pne0000038 (Accessed: April 5, 2022).

VICKERY W. L. & BIDER J. R. 1981. The influence of weather on rodent activity. *Journal of. Mammalogy.* Oxford University Press. UK. **62**: 140-145. doi: 10.2307/1380484 (Accessed: April 5, 2022).

VIVANCO P., ROL M. A., MADRID J. A. 2010. Temperature cycles trigger nocturnalism in the diurnal homeotherm Octodon degus. *Chronobiology International*. Informa PLC**.** UK. **27**(3): 517-534. doi: 10.3109/07420521003743660 (Accessed: April 2, 2022).

WILLIAMS S. E., SHOO, L. P., ISAAC J. L., HOFFMANN A. A., LANGHAM G. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biol.* Public Library of Science. USA. **6**: e325. doi: 10.1371/journal.pbio.0060325 (Accessed: April 1, 2022).

Vrabie Valeria, Ciochină Valentina
The Institute of Physiology and Sanocreatology,
1 Academiei str., Chișinău, Republic of Moldova.
E-mails: valvrabie@yahoo.com; valentina.ciochina@gmail.com

Bodorin Cornelia,
The Institute of Emergency Medicine,
1 Toma Ciorba str., Chișinău, Republic of Moldova.
E-mail: bodorincornelia72@gmail.com

Received: April 15, 2022
Accepted: July 17, 2022