Comparison of natural and pharmacological torpor in homeothermic animals: determination of the activation energy of metabolism

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Abstract

We analyzed the correspondence of the activation energy of metabolism (E) in rats under pharmacological torpor and hypothermia to the findings of the WBE-theory of ecology based on the studies of hibernating mammals: true hibernators and daily heterotherms. We found that in rats in a state of pharmacological torpor lasting about a day, E was close to that of daily heterotherms, while in anesthetized rats with hypothermia lasting for several hours, E was significantly lower, which is in sharp contradiction with the theory. We have shown that in rats classified as homeotherms, at short-term hypothermia the changes in metabolic rate precede the changes in body temperature by the interval Δt. We hypothesized that in poikilotherms, changes in metabolic rate may lag changes in body temperature by (-) Δt. Given this time shift, we proposed an approach to E correction in order to minimize its deviation from theoretical predictions.

Introduction

Hypothermia achieved by physical and/or pharmacological cooling is widely used in medicine at cardiac arrest, ischemic or traumatic injury of different organs . In addition, the state of pharmacological hibernation or synthetic torpor, accompanied by a decrease in body temperature and by suppression of metabolic activity, is supposed to be used for long-term space travel .

Most mammals, known as homeotherms, are able to maintain a constant body temperature throughout their lives. However, during the winter cold period, which is characteristic of high latitudes, or the lack of water and food in the desert, some mammals, called heterotherms, have acquired the ability to adapt by reducing the body temperature. Analysis of the metabolism of heterotherms allowed dividing them into two large groups: daily heterotherms, who are able to be in a state of torpor for hours, and true hibernations, capable of being in a state of torpor for many days and even months . Due to reduced metabolic rate, hibernating animals have increased resistance to adverse environmental factors, including low temperatures, lack of food and water, and long-term mobility restriction .

Studies of the dependence of metabolic rate on temperature are an important part of theoretical biology, namely metabolic theory of ecology (MTE). It has been further developed by West, Brown, and Enquist and known at present as WBE-theory and now tested on a wide variety of plants and animals from unicellular to higher organisms . According to the principles of allometric scaling proposed by WBE-theory, the metabolic rate depends on two variables: body mass and body temperature according to equation :

$I = i_o \ M^{\,3/4} e^{-E/kT}$ (1)

Symbols used: M – body mass, E – activation energy of metabolism, E_c - corrected activation energy of metabolism, k – Boltzmann constant, T – body temperature in Kelvin degrees.

Note: the body mass (M) is taken with exponent 3/4 in accordance with Kleiber's law and used in the model of allometric scaling of body sizes .

For the convenience of analysis, it was suggested to use the logarithmic expression of the above formula:

$ln(I/M^{3/4}) = - E(1/kT) + ln(i_o)$ (2)

This expression is a linear equation. The slope coefficient of this straight line (E) corresponds to the metabolic activation energy, which is assumed to be in the range of -0.6 eV – -0.7 eV for all organisms from unicellular to giant mammals and plants . The mathematical expression $ln(IM^{3/4})$, sometimes called the mass corrected metabolic rate, reflects the energy released by the body during aerobic respiration, which in mammals significantly exceeds the heat output from anaerobic processes. Therefore, we can consider this expression as an assessment of the body's internal heat production. When analyzing the temperature dependence of E for homeothermic animals, with the fact that body temperature of these animals is normally kept constant, appropriate measurements of Ein most species of mammals and birds are problematic. Therefore, for such an analysis, data on hibernating species are used, whose body temperature can fluctuate significantly under natural conditions.

Recently, we have developed a composition of drugs capable of initiating a long-term and stable pharmacologically induced torpor-like state (PITS-composition). In experiments on rats, it was shown that after intravenous injection of the PITS-composition, there was a 16–17-hour decrease in body temperature by 7–8 ^{o}C at the ambient temperature of about 22^{o}C to 23^{o}C. Then, the animals came out of torpor, and their body temperature returned to the original level without the application of external heating. In this work we aim at analyzing E in order to determine to what extent hypothermia induced in rats with PITS-composition is similar to that in naturally hibernating animals.

Materials and Methods

Wistar rats (male, age 2–4 months, weight 200–250 g) were delivered from Stolbovaya Breeding Farm (Russian Federation) and kept in standard conditions. Animal care was performed according to the guidelines established by the European Council Directive 2010/63/EU and in accordance with the "Regulations for Studies with Experimental Animals" (Decree of the Russian Ministry of Health of 12 August 1997, No. 755). The protocol was approved by the Commission on Biological Safety and Ethics of the Institute of Cell Biophysics, Russian Academy of Science. The light cycle was 12 h light and 12 h dark. The air temperature was maintained at about 21 °C to 22 °C. A standard diet and water were provided ad libitum. All animals were delivered in the experimental room in no less than two hours before the beginning of the experiment.

Preparation of PITS-composition

The procedure for preparing the PITS-composition was described earlier . Shortly, to prepare the composition we used the following substances obtained from Sigma-Aldrich and PMBio: 1. Propranolol hydrochloride 5 mg/kg of animal weight; 2. Ivabradine hydrochloride 5 mg/kg of animal weight; 3. Diphenhydramine hydrochloride 5 mg/kg of animal weight; 4. Propylthiouracil 5 mg/kg of animal weight; 5. Serotonin hydrochloride 5 mg/kg of animal weight; 6. Magnesium sulfate 30 mg/kg of animal weight; 7. Reserpine 1 mg/kg of animal weight; 8. Periciazine, 4 mg/kg of animal weight; 9. Lipofundin® MCT/LCT 20% 2.0 ml/kg of animal weight. 10. The emulsion was saturated with inert gas xenon 0.54 ± 0.09 ml/ml (gas/liquid).

The procedure of injection in rats

As it was described in detail earlier , PITS-composition was injected through catheter. Rats were catheterized in the jugular vein and were able to freely move in a system with swivels (DiLab 100,014, USA). One day before the experiment, 100 μl of heparin (50 IU/ml) was injected into the catheter. On the next day, 1 ml of PITS-composition or vehicle (physiological solution) was injected into the catheter in accordance with

protocol of the experiment. After the injection of drugs, the catheter was filled with 100 μl of heparin (50 IU/ ml) to flush the system and prevent clotting in the catheter.

Xylazine (Interchemie, Netherlands), 2% dissolved in physiological solution, was administered intramuscularly (0,40 ml per 1 kg body weight).

Measurement of physiological parameters

Core body temperature was measured by rectal sensor RET-2 in rats (Physitemp, USA, ± 0.1°C). Measurement of metabolic parameters (oxygen consumption, carbon dioxide release, respiratory coefficient and energy release) was carried out using mm-100 metabolic monitoring system (CWE Inc., USA). Animals were housed in the chamber of 7.5 L. The rate of air flow was 1500 ml/min. The monitoring was carried out at room temperature in the specified time intervals including 3 min for the measurement.

We also used literature data on oxygen consumption by hibernating animals . In the calculations we assumed that in animals 1 ml of inhaled oxygen yields 20.1 Watt .

Statistics

Linear regression to experimental points, determination of SD andr^2 were carried out in software OriginPro (OriginLab Corporation, USA). Values were presented as mean ± SD. Statistical analysis of the reliability of differences in the data was carried out using One Way ANOVA nonparametric Tukey's multiple comparison test in GraphPad Prizm 7 (GraphPad Software Inc., USA).

Results

It has been shown previously that after injection of the PITS-composition, the rat body temperature decreased by about 7.5 @C, while the half-width of the temperature curve was about 16.5 h, at ambient temperature of 22@C to 23@C (Fig.1). For comparison, we investigated the effect of anesthetic xylazine, which is also able to reduce the body temperature of animals, often in combination with ketamine . In our experiments, xylazine initiated a decrease in the rat body temperature by about 5.5 @C, at ambient temperature of 22@C to 23@C.The half-width of the temperature curve was about 3 hours (Fig.1).

It has been shown previously that after intravenous injection of PITS-composition, there was a reversible decrease in metabolic rate and body temperature. Both parameters changed almost simultaneously (Fig. 2). We used these data to compare E in a pharmacological and natural torpid state (Fig.3). It has been found (Fig.3) that in the pharmacological torpor lasting typically one day in rats (Rats-PITS),E = -0.56±0.03 eV which was close to the corresponding value in daily heterotherms E = -0.57±0.04 eV. In true hibernators, this value was significantly higher (E = -0.80±0.04 eV), while in anesthetized animals it was significantly lower (E = -0.17±0.071 eV). In addition, in rats treated with xylazine, the small value of the coefficient of determination (r^2 = 0.12) indicates a wide spread of the experimental points and poor quality of the regression model. Therefore, it is necessary to evaluate in more details how the metabolic rate depends on the temperature during anesthesia with xylazine.

The analysis shows (Fig.4A) that in the presented experimental data obtained on rats anesthetized with xylazine there was a time lag between the temperature curve and the heat production curve. The question arises whether this lag could be a reason for low E andr^2 values? We have found that a significantly better coincidence of the curves was observed when the heat production curve shifted by $\Delta\tau$ = 1 hour (Fig.4A). In addition, to confirm this assumption, we performed a numerical shift of the heat production data (Fig. 4B) and find the imaginary dependence of E on the shift (Fig.4C). The minimal E was observed at the shift $\Delta\tau$= 1 hour and considered the corrected E_c=-0.67±0.11 eV, which in this experiment corresponded to two intervals between measurements: Shift +2 (Fig.4 D). It should also be noted that as a result of the shift, the coefficient of determinationr^2 increased significantly from 0.12 (Fig.3A) to 0.70 (Fig.4 C), which indicates an improvement in the model of regression analysis.

Since in homeothermic animals, the change in the body temperature occurs as a result of changes in heat production, the lag between the temperature and the heat production is associated with a limited rate of the

body heat conductivity and heat dissipation. Therefore, it takes a time ($\Delta\tau$) to achieve a balance between the metabolic rate and the body temperature. This nonequilibrium situation could be the reason of an incorrect estimation of E . The correctedE_c = -0.68±0.17 eV obtained at the imaginary numerical Shift+2 (Fig.3) is significantly ($p < 0.0001$) larger than that, obtained (Fig.3), in daily heterotherms (E = -0.57±0.04 eV) and in artificial hibernation, lasting for a day (E = -0.56±0.03 eV), but smaller than that in true hibernators (E = -0.8 eV), hypothermia of which lasts for weeks and even months.

The influence of the imaginary shift on the E was studied on rats after injection of the PITS-composition (Fig. 5). In this case, the minimal E was obtained without any shift (Shift 0), which indicated the state of equilibrium between heat production and heat dissipation during pharmacological torpor, which was significantly longer than that in anesthesia.

Discussion

In this paper, we investigated the activation energy of metabolism$E$ in rats after injection of a previously developed PITS-composition capable of inducing a daily hypothermia and torpor-like state in rats , as well as in rats after injection of anesthetic xylazine, which can reduce body temperature for a few hours. The use of this anesthetic is based on the fact that it, often in combination with ketamine, can induce hypothermia in animals whereas dexmedetomidine, an analog of xylazine, can be used to initiate hypothermia in humans, and is proposed for protection in the emergency cases or during long-term space travels .

Data on changes in body temperature and oxygen consumption by rats after injection of PITS-composition or xylazine were used to determine$E$ by the linear regression slopes with regard to mass-corrected metabolic rate $\ln(I/M^{3/4})$, which was equivalent to heat production, and the inverse body temperature $1/kT$. In accordance with the WBE-theory, E should be in the range of -0.6 – -0.7 eV. For example, in hibernating mammals E = -0.69 eV . Our calculations made separately for daily heterotherms and true hibernators revealed some deviations from the above range: in daily heterotherms E = -0.57±0.04 eV, and in true hibernators E= -0.80±0.04 eV. This deviations from a classical viewpoint is not unexpected, especially in the studies of poikilothermic organisms. For example, in the study of fish, E = - 0.5 eV . Even lower values were obtained for the marine copepod , rocky-shore eulittoral-fringe snail (*Echinolittorina malaccana*) that experiences fluctuating temperatures , while the teleost fish study revealed excessively large values of E = -0.79 eV .

The presented above significant deviations in E are inexplicable from the point of view of the WBE-theory , which begins with the observation that temperature controls metabolism through its effect on the rate of biochemical reactions. It is known, that the reaction kinetics depends on temperature according to the Boltzmann factor. In line with Clarke's criticisms, while statistical thermodynamics provides a very successful description of the behavior of a simple system where temperature is the only variable that changes, organismal metabolism is very different. Organismal metabolism involves a large number of physiological processes, each of which interacts with many others . Although it is generally accepted that changes in temperature should lead to corresponding changes in metabolism, which was demonstrated in model systems or in mitochondrial suspension, the question is how universal the application of this physical principle to processes at the level of multicellular organisms .

We have found that E in rats injected with PITS-composition was close to the corresponding value in natural daily heterotherms, but smaller than that in true hibernators. This suggests that the PITS-composition is able to initiate in homeothermic organisms, like rats, a state close to daily heterotherms, which, however, differ from true hibernation. Indeed, the state of pharmacological torpor that occurs in rats after a single injection of the PITS-composition lasts for about 16 hours, which is characteristic of daily heterotherms rather than of true hibernators, experiencing the state of torpor and hypothermia during many days and even several months .

When rats were injected with the anesthetic xylazine, their body temperature also decreased for several hours (the curve half-width = 3 h), however, E was about three-fold less than that after injection of PITS-composition (E = -0.17±0.071 eV) and did not correspond to the value for a natural state of hibernation .

What can be the reason for such a significant difference?

For determination of the resting metabolic rate and correct assessment of E , a stationary metabolic state of animals has to be achieved . In our experiments, the body temperature of animals after injection of the anesthetic xylazine constantly changed: initially decreased, and then increased followed by significant changes in heat production and, accordingly, the animal state could not be regarded as stationary. Therefore, although the anesthetized animals in our experiment were immobile, their metabolism cannot be regarded as the resting metabolism. In homeothermic animals, the body heat production is the source for the elevated body temperature compared to the ambient one. The metabolism plays a leading role in maintaining the body temperature of warm-blooded animals in our understanding of the mechanisms of homeothermy. Thus, changes in body temperature lag behind changes in metabolic rate and, accordingly, the changes in oxygen consumption. Lag compensation by means of an imaginary numerical shift of oxygen consumption by 1 hour allows us to obtain corrected E_c = -0.68 eV, close to the corresponding values for hibernating mammals .

In experiments with PITS-composition, the body temperature initially decreased and then stabilized for a long period of time in a state of hypothermia followed by restoration of the initial temperature level. Thus, in animals under PITS the period of steadily lowered body temperature accounted for a significant part of the time of pharmacological torpor, and can be regarded as a stationary resting state. Oppositely, in anesthetized animals during short-term hypothermia a stationary state is not achieved.

As mentioned above, in the study of poikilothermic organisms, there may be significant variability of E . It can be assumed that in this case, there may also appear a temporary mismatch in temperature and metabolic rate. Since in the cold-blooded organisms the ambient temperature is the main factor determining the body temperature and accordingly the metabolic rate, we suppose that the changes in metabolic rate may occur later than the changes in body temperature for both physiological reasons, including the time necessary for changes of heart rate, respiratory rate, blood vessel conductivity, etc. and even slower metabolic changes in composition of membrane lipids leading to a decrease in phase transition temperature of lipids which is coupled with enhanced cold induction of genes . Thus, if for homeotherms the time lag of temperature behind metabolism was designated as $\Delta\tau$, then for poikilotherms the lag of metabolism behind temperature should be denoted by the opposite sign, as (-)$\Delta\tau$. We assume that as in a case with homeotherms mentioned above, for poikilotherms the correct calculation of E should be made taking into account the time shift mentioned above .

Conclusions

Metabolic theory of ecology, being a significant part of theoretical biology, considers the transformation of energy by organisms. In accordance with the WBE-theory, E should be within the range of -0.6 – -0.7 eV which is often not supported by the experimental data. We have found that in homeothermic animals, such as rats, under pharmacological torpor the standard methods for determining E are effective only when changes in the body temperature occur not less than within a day. When changes occur within hours, this case is typical of animals under anesthesia, it is necessary to take into account a time lag ($\Delta\tau$) of body temperature behind metabolic rate. Since in homeothermic animals, the changes in the body temperature result from changes in heat production, the lag of temperature behind heat production is associated with a limited rate of the body heat conductivity and heat dissipation. Therefore, it takes a time ($\Delta\tau$) to achieve a balance between the metabolic rate and the body temperature.

We suggest that in poikilotherms, the changes in metabolic rate may lag behind the changes in body temperature for a period (-) $\Delta\tau$. It may happen when the ambient temperature changes sufficiently rapidly, within a few hours. In this case, (-) $\Delta\tau$ should also be taken into account when calculating E . The reasons for the temporary mismatch of changes in temperature and metabolism in poikilotherms can be both physiological: the time required for changes in heart rate, respiration, vascular conductivity; and the well-known biochemical ones: temperature control of the lipid composition and fluidity of the membranes, which are regulated by the appropriate genes.

Acknowledgments

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Figure legends

Fig.1. The effect of PITS-composition (n = 7) and xylazine (n = 5) on T_b of rats at the ambient temperature of 22@C to 23@C. In the experiment, no additional means were used to accelerate the animal cooling or heating. Mean ±SD is presented. Drugs were injected at zero time, as indicated by arrow (Inj).

Fig.2. Changes in body temperature and heat production in rats after injection of PITS-composition. The injection was performed at zero time, as indicated by arrow (Inj). Metabolic rate (I) was determined as described in the section of Materials and Methods. The body mass (M) is taken with exponent 3/4 in accordance with Kleiber's law and requirements of WBE-theory (see Eq. 1).

Fig.3. Determination of E in electron volts (eV) for different animals. A – Relationship between mass-corrected heat production, $\ln(IM^{3/4})$, measured in watts/g$^{3/4}$, and temperature, $1/kT$, measured in K. The overall slopes estimate the activation energy E in electron volts in rats (n=6) after injection of PITS-composition (Rats-PITS) and after injection of the anesthetic xylazine (Rats-Xyla) obtained from our measurements, and also in daily heterotherms (n=31) and true hibernators (n=123) obtained elsewhere . B – E for the specified groups of animals represent the slopes of straight lines on the panel A. For pair: Rats-PITS and Heterotherms, E was not statistically different. There is a significant statistical difference in E for both Rats (Xyla) and Hibernators compared to those for pair Rats-PITS and Heterotherms, $p < 0.0001$. Note: r^2 – coefficient of determination.

Fig.4. The dependence of E on a numerical time shift of the heat production curve. Rats were anesthetized with xylazine. A – A shift of the heat production curve by 1 hour is shown; 1 hour corresponds to 2 intervals between measurements: Shift +2. B – Example of implementation of numerical shifts of heat production values by the specified number of intervals between measurements. C – The effect of the shifts on$E$, which corresponds to changes in the slopes of the regression lines. D – Dependence of E on the number of intervals of the numerical shift (Shift).

Fig.5. Rats in pharmacological torpor after injection of PITS-composition. A – An example of the dependence of heat production on the reverse temperature. Compare it with the graph presented in Fig.4C. B – The dependence of E on the Shift (Compare it with the graph presented in Fig. 4D). The data were obtained from the experiments (n = 5) similar to that presented in Fig.2. Note: r^2 – coefficient of determination.

Figure 1

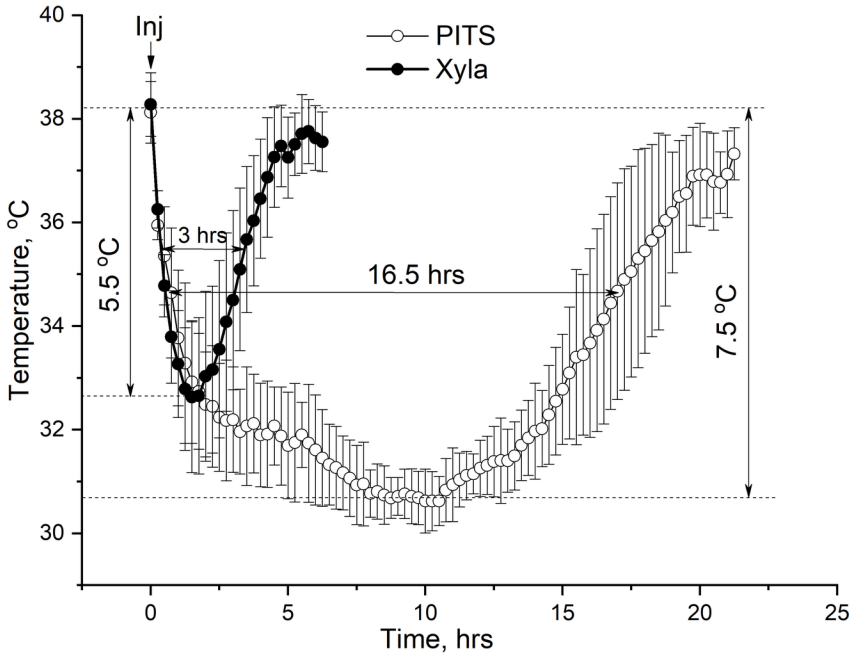


Figure 2

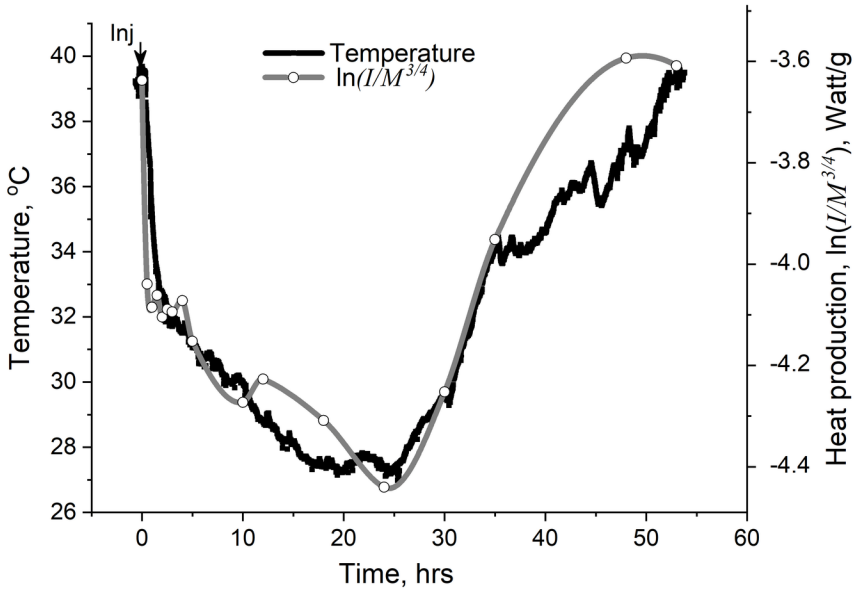


Figure 3

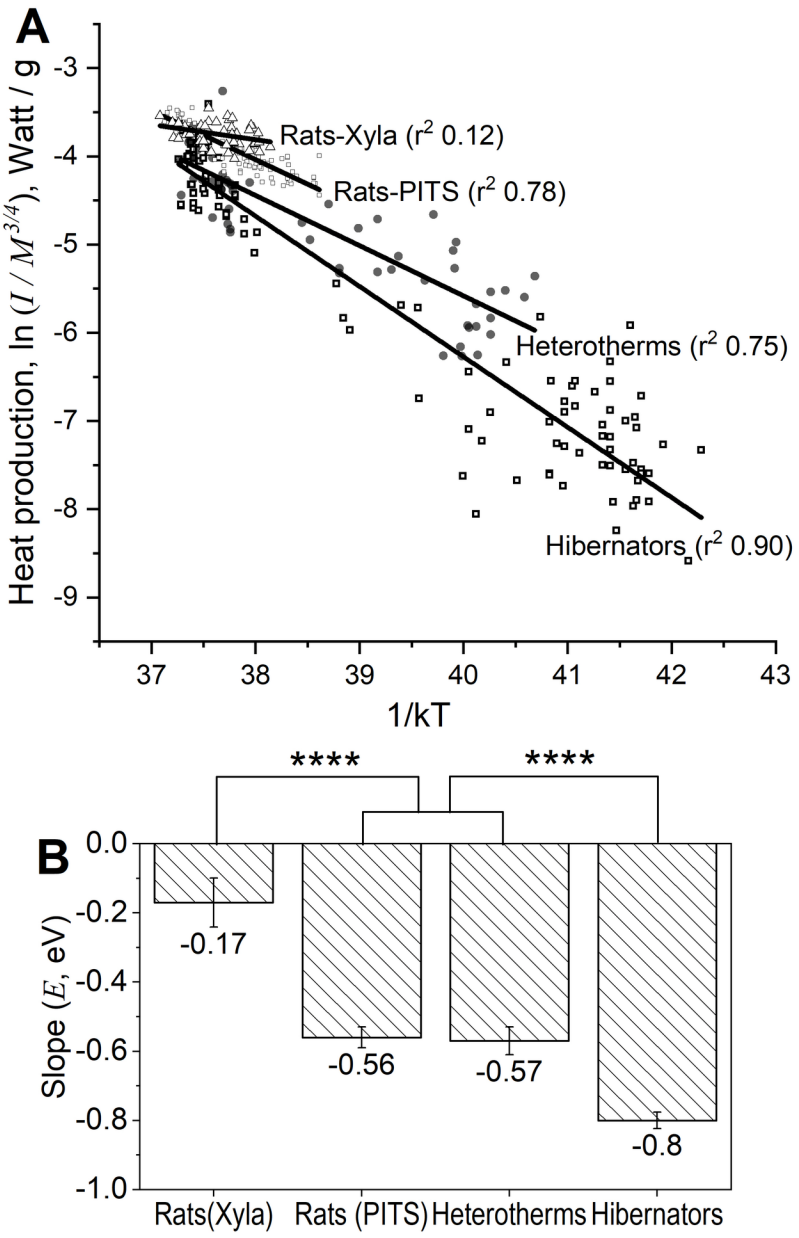
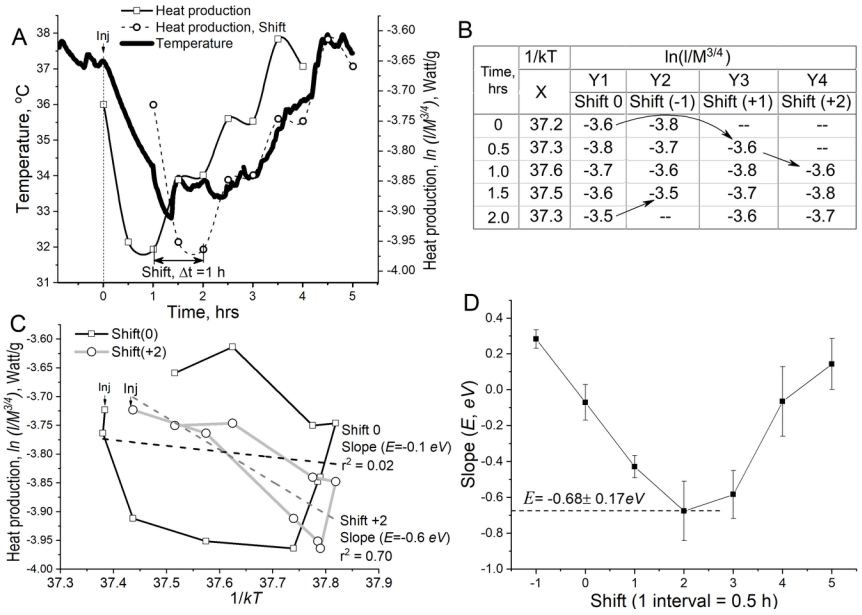


Figure 4



Time, hrs	1/kT	ln(I/M$^{3/4}$)			
	X	Y1	Y2	Y3	Y4
		Shift 0	Shift (-1)	Shift (+1)	Shift (+2)
0	37.2	-3.6	-3.8	--	--
0.5	37.3	-3.8	-3.7	-3.6	--
1.0	37.6	-3.7	-3.6	-3.8	-3.6
1.5	37.5	-3.6	-3.5	-3.7	-3.8
2.0	37.3	-3.5	--	-3.6	-3.7

Figure 5