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Thermally Dependent Behaviour of Cold-Blooded Animals: Overcoming Two Temperature Barriers on the Way to Warm-Blooded

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Abstract

Background: The temperature preferences of cold-blooded animals are different for different groups of animals and are closely related to their evolutionary status and level of adaptive capabilities. The range of preferred temperatures for reptiles (28˚C - 32˚C) coincides with the zone of temperature-dependent increase in the rate of the M-cholinergic reaction in the brain, which was previously found in warm-blooded neurons. The growth of the M-cholinergic process contributes to the transition of adaptive behavior to a higher level. Of the cold-blooded, only reptiles actively use thermoregulatory behavior as a tool to achieve a temperature optimum. This paper is devoted to the study of the behavior of red-eared turtles aimed at reaching the range of preferred temperatures. Temperature conditions necessary for the survival of different groups of cold-blooded animals are compared and the reason for temperature preferences is discussed. **Methods:** The behavior of turtles was studied under conditions of variable solar radiation and in a 3-section terrarium with gradual temperature ranges: 23˚C - 24˚C; 28˚C - 31˚C and 45˚C - 50˚C. **Results:** It was found that prolonged (up to 1.5 hours) heating at a temperature of 45˚C - 50˚C was the best way to achieve the preferred temperatures. This method of heating results in increasing the temperature of the shell to an average of 37˚C, followed by a very slow decrease during 4 - 6 hours within the limits that closely coincided with the preferred level. **Conclusion:** The results obtained demonstrate that the main function of the turtle shell is to accumulate heat and keep it for a long time in a variable climate. The preferred temperature achieved in this case is necessary for the temperature-dependent transition of the rate of the M-cholinergic reaction in the brain to a higher level, which creates more significant adaptive capabilities. The temperature range of the M-cholinergic process has two inflection points at 27˚C - 29˚C and 34˚C - 36˚C. In accordance with these values, the temperature preferences of cold-blooded animals are divided into three groups: 0˚C - 28˚C (fish and amphibians); 28˚C - 32˚C (reptiles) and 34˚C - 36˚C (dinosaurs). Different ranges of preferred temperatures correspond to three stages of adaptive development.

Keywords

Preferred Temperatures, Adaptive Capabilities, Turtles, Shell, Ice-Free Era, Permian Period, Mesozoic Era

1. Introduction

Different groups of inhabitants of the Earth are characterized by different temperature ranges in which their normal activity can be realized. The widest temperature range (about 25˚C - 28˚C) begins around 0˚C—it is typical for fish, amphibians and a large part of invertebrates. For reptiles and insects, the range of normal active life is much narrower: it starts at an average of 20˚C and is continued till 30˚C - 34˚C. Warm-blooded animals, among which there are no invertebrates, are satisfied with only a few degrees of comfortable existence, which in different species proceeds at different (from 30˚C to 41˚C), but always constant temperatures [1] [2].

For the first group of animals, due to the wide temperature range of their normal functioning, and for warm-blooded animals warmed by the heat of internal metabolic origin, there is practically no need to take care of body temperature. Reptiles and insects are of another matter: we can remember how lizards on a sunny May Day bask on the steps of the porch heated by the sun, or butterflies—at sunset, opening their wings towards the sun's rays. Moreover, all of this is continued for a long time, fearlessly, despite the fact that this secret passion is open to prying eyes. Conceivably, the body temperature is the most obligatory condition for the realization of any type of activity.

Within the temperature range when reptiles are active, there is a range of temperatures at which the animals tend to stay most frequently. Such temperatures are called preferred. The level of preferred temperatures for all reptiles is quite high: from 28˚C to 32˚C. For different species of terrestrial turtles, the preferred temperatures somewhat vary from 28.5˚C to 29.8˚C, for the gecko—27.5˚C - 33˚C. For Central Asian turtles under laboratory conditions, the preferred temperature range is set at 26˚C - 28˚C [3]. Upon careful consideration of the presented values, it can be seen that the values of preferred temperatures in reptiles are very close. They necessarily pass through the 28˚C point and extend up to 30˚C - 32˚C. This means that in the temperature range of 28˚C - 32˚C, stable functional changes occur in the organisms living at this temperature. Such changes should create evolutionary advantages for reptiles in comparison with animals whose existence passes at a lower temperature range (0˚C - 28˚C).

On surviving slices of the sensorimotor cortex of guinea pigs, it was found that when the incubation medium is cooled below 32˚C - 34˚C, the frequency of spontaneous firing of neurons, practically unchanged up to 30˚C, steadily decreases at a temperature of 27˚C - 29˚C [4], while the range of its variability also decreases. The importance of this circumstance lies in the fact that the regulation of spontaneous activity by brain neurons is a necessary condition for the implementation of adaptive activity in all its diversity: from the perception of the surrounding world to the formation of adaptive motor responses [5] [6]. Due to the fact that the diversity of habitat conditions for turtles [7] [8] is much wider than for amphibians and fish, it should be concluded that the adaptive capabilities at a temperature of 20˚C - 34˚C are richer than in the range of 0˚C - 28˚C. Therefore, the temperature-dependent increase in the frequency of spontaneous firing of neurons is exactly that circumstance that brings reptiles to a higher evolutionary level. Preferring a temperature of 28˚C - 32˚C for their habitat, reptiles provide a high adaptability of behavior under the conditions of a new evolutionary advantage, the beginning of which was associated with climate warming on Earth during the onset of the Permian geological period [9]. The advantages acquired in this way force the reptiles to constantly carry out thermoregulatory behavior.

Thus, reptiles are a convenient object not only for demonstrating thermodependent behavior, but also for revealing the evolutionary development of the adaptive function of the brain.

This work is devoted to the study of the thermo-biological behavior of red-eared turtles (*Trachemys scripta elegans*) under conditions of a wide (23˚C - 50˚C) temperature diversity of the external environment. On the basis of own and literature data, the range of preferred temperature characteristics of reptiles is substantiated, and the ways used by turtles to achieve optimal temperature are determined. A relationship is postulated between the temperature of the environment of cold-blooded animals and their adaptive capabilities.

2. Methods

Experiments on the study of thermo-biological behavior were carried out on 11 turtles (*Trachemys scripta elegans*) of different age, sex and weight. When handling animals, the authors followed recommendations described in Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. At Moscow State University (MSU), compliance with the standards for conducting research on animals is monitored by the Commission on Bioethical Ethics, created by order of the Rector of MSU No. 144 dated March 5, 2008; GOST 33215-2014 “Guidelines for the maintenance and care of laboratory animals, rules for equipping premises and organizing procedures”; GOST 33219-2014 |Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of fish, amphibians, reptiles|.

Five turtles were used for summer experiments in a transparent plexiglass terrarium to study the role of solar radiation on animal behavior, and 6 turtles were used in winter experiments, where in a 3-section terrarium, by creating a temperature gradient in a wide range (from 23˚C to 50˚C) it was supposed to study what temperature conditions turtles prefer and how their thermo-dependent behavior is formed. Both terrariums were provided with room temperature water cuvettes and were large enough for turtles to move freely.

In summer experiments, which were carried out in June or September, natural sunlight was used throughout the daylight hours. In the morning and evening hours, the terrarium where the turtles were kept was not illuminated by the sun's rays, while in the daytime the wall of the terrarium facing the sun was constantly under the influence of solar radiation.

In winter experiments, natural lighting was not provided. All three compartments into which the terrarium was divided were individually permanently lit with 15 W energy-saving lamps. The compartments of the terrarium were separated by partitions, the central part of which was intended for the passage of turtles from one compartment to another. The transition between the compartments was closed with curtains made of narrow Plexiglas plates, which did not create obstacles for the movement of the turtles. Each compartment had its own temperature. The temperature of the first compartment corresponded to the room temperature typical for January, February, March and was 23˚C - 24˚C. The temperature value in the first compartment coincided with the minimum voluntarily tolerated temperature for turtles. In the second and third compartments, using a set of ceramic infrared heaters (China), a temperature gradient was created: in the second compartment, the temperature corresponded to the preferred values: 28˚C - 32˚C, and in the third compartment it was at the critical level and even higher: 45˚C - 50˚C [3]. Using non-contact thermometers (Guizhou Xinhui Optoelectronic Technology Co., China), the temperature of the shell was periodically measured, for which the turtle was transferred to the first compartment at the time of measurement.

The movement of the turtles within the terrarium was recorded every minute within 6 - 8 hours of observation and presented graphically, taking into account the duration of turtle's stay in each compartment.

3. Results

3.1. Solar Heating (June, September)

A large experimental plexiglass terrarium with a large pool in which all five turtles could dive at the same time was located on a table opposite the south-facing windows. In the morning hours, the sun's rays did not illuminate the terrarium, and the turtles placed in it freely moved in all directions. After exploring the territory given to them, the turtles, as a rule, plunged into the pool, the water temperature in which, as well as the air temperature, was 28˚C, *i.e.* matched the preference. The addiction to water procedures, which the turtles demonstrated in the aqua-terrarium, their usual living place, they also manifested in the conditions of the experimental terrarium: the reptiles practically did not leave the pool, where they stayed for many tens of minutes.

In the summer months, the height of the solstice is such that the sun's rays from the luminary making its daily journey fall with a slight deviation from the vertical. Therefore, the appeared solar radiation created glare on the wall of the terrarium, which penetrated deep into the terrarium by a very small amount. But this was enough for the turtles to leave the pool, located in the far corner of the terrarium, one by one, and rushed to the sun glare on the wall facing the sun. It was a mesmerizing sight. Approaching the wall of the terrarium, where the sun left its fiery imprint, the turtles turned their backs to it, raised their heads high and froze in immobility. This posture could be maintained for about an hour or even longer. The response to sunlight was fully reproduced in all five turtles the next day, as well as in experiments on individual turtles thereafter. Figure 1 shows several examples of different experiments that demonstrate the length of time turtles stay in the zone of sunlight. The pictures were taken on 3 turtles with intervals of 10 minutes between the pictures of each photo session. The furnishings captured in the pictures indicate that the turtles were in the same place throughout the entire photo session. The sun glare visible on the shells of turtles means that the localization in relation to the sun is chosen by the turtles correctly. The temperature in the area where the sun's rays fell exceeded the average temperature in the terrarium by 0.5˚C - 1.5˚C. The duration of stay in the illuminated zone of turtles 1 and 2 was 30 minutes, of turtle 3 with 1 hour 10 minutes (Figure 1). However, in different experiments under the same temperature conditions, prolonged sunbathing was not observed in all turtles. Some turtles

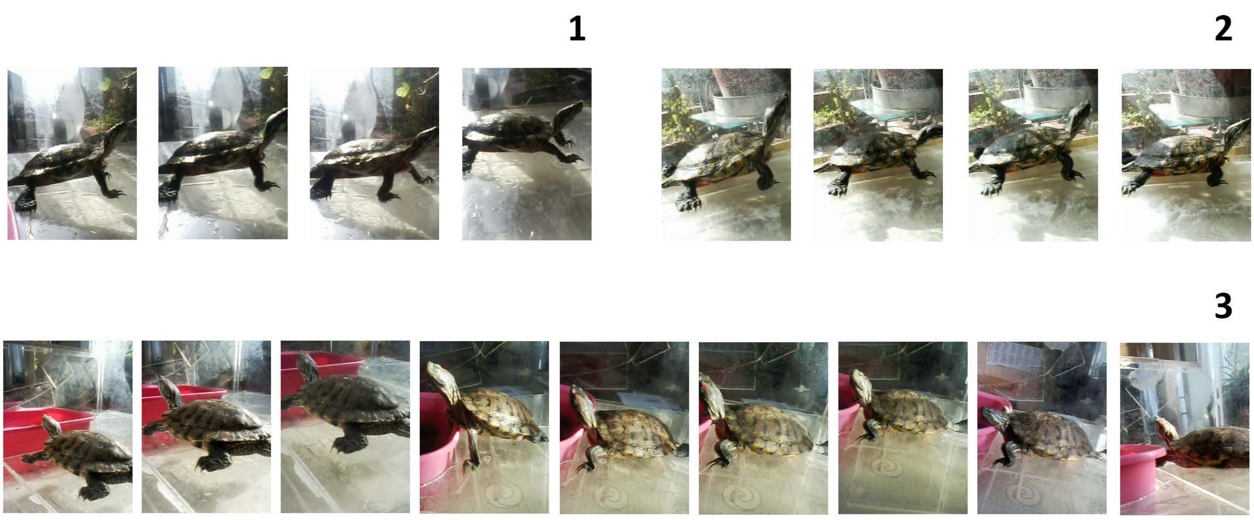

Figure 1. Locations of three turtles (1 - 3) during solar heating. Each photo of each photo session was performed 10 minutes after the previous one. Observations on turtles 1 and 2 thus lasted 30 minutes, on turtle 3 lasted 1 hour and 20 minutes. Explanations in the text.

preferred constant exploratory movements in the terrarium (shuttle behavior). Heating with solar radiation is a natural behavior for all reptiles and flying insects. Surprisingly was an additional heating occurred in turtles at a preferred temperature—a natural summer temperature of 28˚C. The steady tendency to heat up at this temperature, with a very small additional excess of 1.5˚C in the solar glare, indicates that the temperature-dependent behavior is determined not only by the current temperature needs. An attempt to establish the cause of additional heating in the zone of preferred temperatures was made in winter experiments.

3.2. Gradual Heating (January-March)

In the second series of experiments, carried out in the winter-spring time, 6 turtles participated. Their temperature preferences were tested individually in a three-section terrarium with set temperatures of 23˚C - 24˚C; 28˚C - 32˚C and 45˚C - 50˚C during 5 - 8 hours of observation. Turtles were used repeatedly in the experiment at intervals of about one week. The shell temperature was periodically measured using a non-contact thermometer. Each experiment began by placing the turtle in the compartment with the lowest (room) temperature. Animals were given the opportunity to move from one compartment to another and stay in each of them for any time. Turtles, as a rule, quickly adapted in the experimental terrarium and began to actively move in all three compartments.

Figure 2 shows the 5-hour movement of one of the turtles between areas with different temperatures. The different heights and shading of the columns indicating the 1-minute stay of the turtle in one of the compartments demonstrate a fairly regular visit to all three temperature zones. The turtle does not avoid even the hottest compartment, the temperature of which (45˚C - 50˚C) exceeds the upper limit of tolerance. Stay in the first compartment with a room temperature of 24.2˚C is reduced over 5 hours from 41 minutes to 13 minutes, and visits to compartments with a higher temperature gradually increase from 19 minutes in the first hour to 49 minutes in the fifth hour of the experiment. Increasing duration of visits to compartments 28˚C - 32˚C and 45˚C - 50˚C leads to additional heating. As a result, the initial shell temperature, coinciding with the temperature in the first compartment, gradually increases from 24.2˚C to 29.2˚C after 5 hours of observation. This type of thermoregulatory behavior, called shuttle behavior, was recorded in three different turtles in three experiments. Therefore, the shuttle behavior allows the shell to be heated to a temperature close to the preferred one.

Significantly more often (in 11 of 14 experiments), turtles preferred a different method of heating. Placed in a compartment with room temperature (23˚C - 24˚C), they, moving around the entire terrarium, stopped for a long time in the compartment with the highest temperature (45˚C - 50˚C). Despite the fact that the preferred temperature was reached in the compartment of 28˚C - 32˚C, most of the turtles already in the first hour of the experiment chose a very high tem

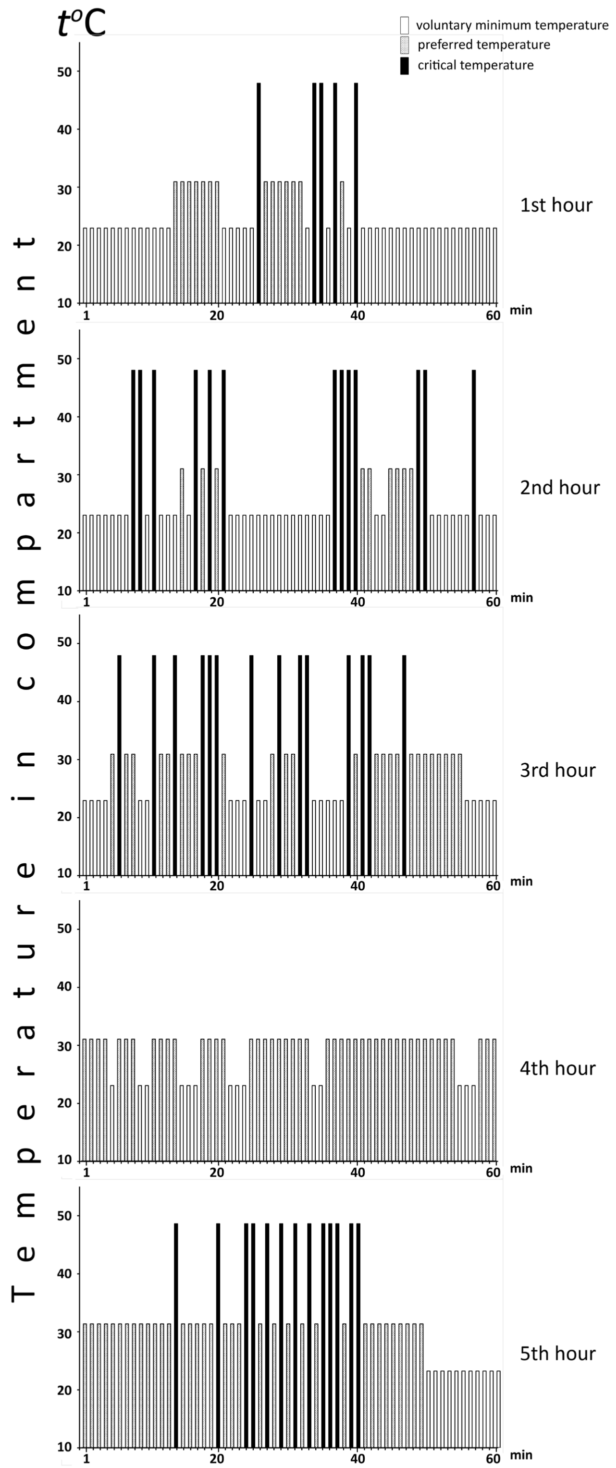

Figure 2. Moving the turtle between compartments with different temperatures in the mode of shuttle behavior. 5 consecutive hours of observation (1 - 5) are represented. Each column on the histograms depicts a 1-minute stay of the turtle in one of the compartments: 1st compartment—room temperature (23˚C - 24˚C); 2nd compartment—temperature 28˚C - 32˚C; 3rd compartment—temperature 45˚C - 50˚C. Horizontally—time, minutes. Vertically—the temperature of the compartment in which the turtle is present at the moment. The stay compartment is also duplicated by the filling of each column. The label is shown at the top right.

perature, critical for survival (**Figure 3** (1, 2, 4, 5)). All 6 turtles, when tested 2 - 3 times, demonstrated this type of thermo-dependent behavior at least once. In most cases, repeated experiments led to the same result. The duration of continuous stay in the 45˚C - 50˚C compartment ranged from 40 minutes to 1 hour

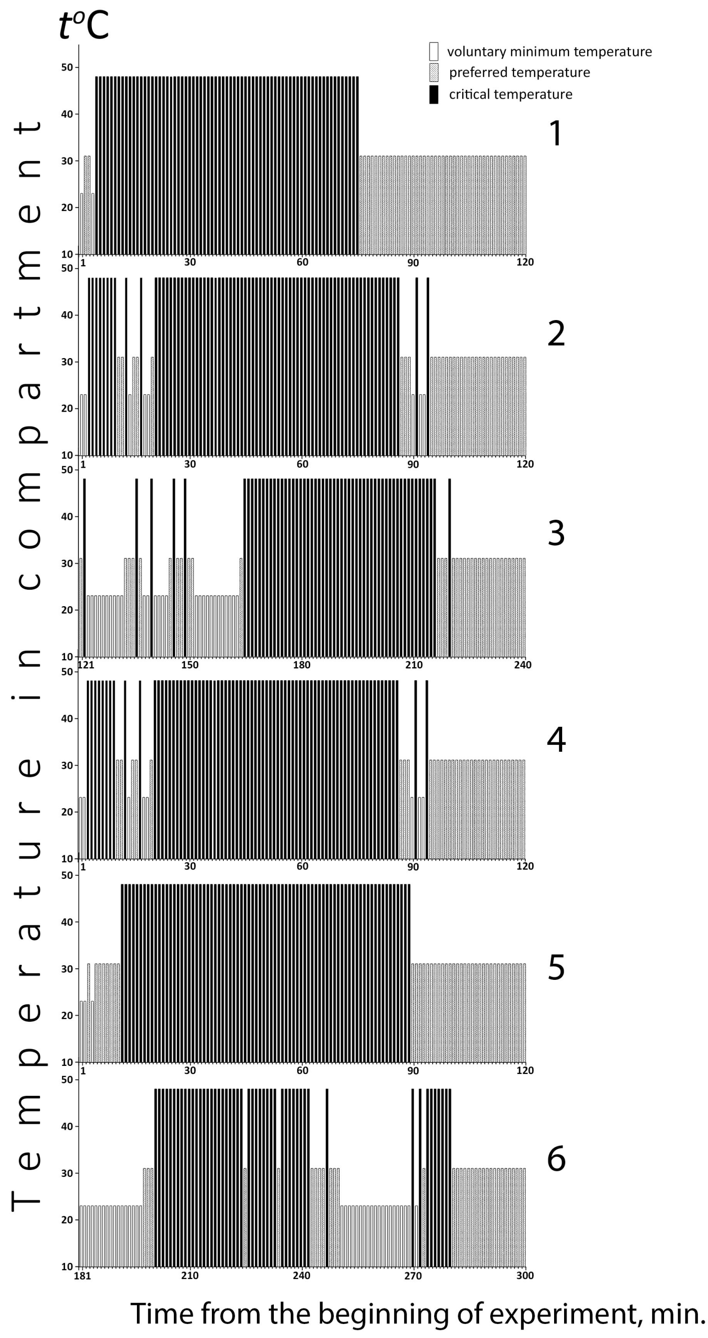

Figure 3. Voluntary choice by turtles of a compartment with a temperature of 45˚C - 50˚C for a long time. All 6 turtles participating in the experiments (1 - 6) are represented. For each turtle from a 6 - 8 hour observation, a 2-hour period is shown when a long-lasting selection of a compartment with a temperature of 45˚C - 50˚C occurs. Horizontally—time from the beginning of the experiment, minutes; Vertically—the temperature of the compartment in which the turtle is at the moment. All designations are as in **Figure 2**.

30 minutes. Animals during this period were inactive, often putting forward their hind limbs. At the final stage, before leaving the region of critical temperatures, in three cases the retraction of the head into the shell was observed. After prolonged heating, the maximum shell temperature was recorded at 39˚C. Long staying in the compartment at 45˚C - 50˚C after its cessation led to a stable selection of compartments with a lower temperature for many hours. And only after 2 - 3 hours the usual shuttle behavior resumed with a visit to all three compartments.

A long stay in the zone of critical temperatures not only led to the heating of the shell to an average of 37.0˚C, but also contributed to maintaining the achieved temperature after leaving the compartment 45˚C - 50˚C. The graphs in Figure 4 for 10 experiments show the dynamics of the shell temperature decrease. It follows from the graphs that even 4 - 6 hours after the prolonged heating period, for most cases, the shell temperature remains above or within the preferred temperature range (in Figure 4 the preferred temperature range is enclosed between two dotted lines). In only one case, the achieved high temperature decreased within one hour. Therefore, the shell can serve as a source of long-term additional heating.

For comparison, the graphs in Figure 5 present data on the shell temperature in cases where the shuttle behavior is the only thermoregulatory one and lasts

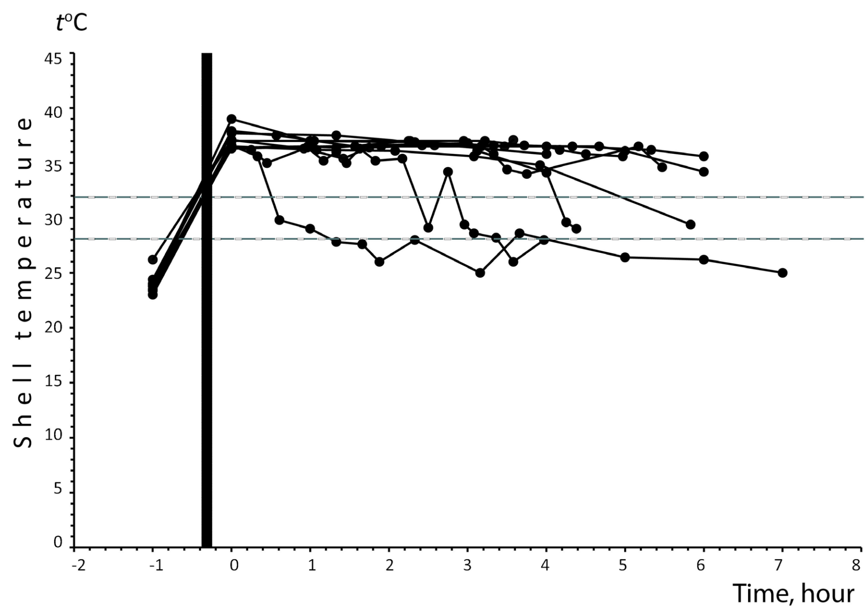

Figure 4. Shell temperature for several hours after a voluntary long stay in a compartment with a temperature of 45˚C - 50˚C. The results of shell temperature measurements in 10 experiments are shown. The abscissa shows the time in hours after a long stay in a compartment with a temperature of 45˚C - 50˚C. The presence of prolonged heating is marked with a black bar before the zero measurement (immediately after the end of heating) of the shell temperature. The initial shell temperature before heating was measured in a compartment with room temperature (23˚C - 24˚C) and marked to the left of zero on the abscissa. The ordinate shows the shell temperature. Two dotted lines parallel to the x-axis mark the range of preferred temperatures.

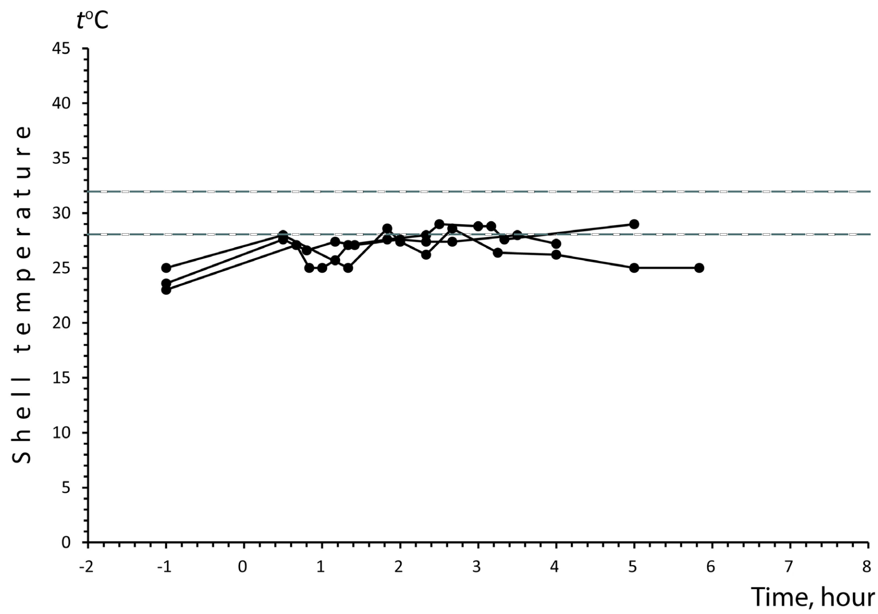

Figure 5. Shell temperature during several hours of shuttle behavior. The results of measuring the shell temperature are shown in 3 experiments on three turtles that showed shuttle behavior in a three-section terrarium. The abscissa shows the time in hours from the beginning of the experiment; the ordinate shows the shell temperature. Two dotted lines parallel to the x-axis indicate the preferred temperature range. The remaining designations are as in **Figure 4**.

for 5 hours. It is clearly seen that although the temperature of the shell increases during the experiment, it is usually lower than the marks characteristic of the preferred temperatures.

Therefore, both in terms of frequency of manifestation and in terms of thermo-biological efficiency, prolonged heating at a very high temperature is preferable to shuttle behavior. Therefore, turtles do not miss any opportunity for additional heating, even if they are at a temperature of 28˚C.

4. Discussion

The appearance of turtles on Earth marked the end of a long period of evolution of life, which took place at constantly changing, but rather low temperatures. Intermittent periods of ice sheet were followed by interglacial epochs over the course of 2.5 billion years ago in the Proterozoic to the Carboniferous-Permian Ice Age, which lasted from 350 to 230 million years ago [10]. All this time, life could only proceed within the lowest temperature range from zero to 25˚C - 28˚C. Therefore, vertebrates: tunicata, agnatha, gnathostomi: chondrichthyes and osteichthyes, as well as amphibia [11], live at these temperatures [2].

For the appearance of reptiles, whose active life takes place in the temperature range from 20 to 30˚C - 34˚C [3], other temperature conditions were necessary. The first reptiles—turtles arose 240 - 220 million years ago [7] [11], which intersects with the retreat of the Carboniferous-Permian Ice Age (230 million years ago [10]) and the development of rapid global warming on Earth [9], which led to the movement of glaciers to high latitudes and a decrease in the planet's albedo. At the same time, due to the formation of mountain belts and ridges on the then unified continent Pangea, along its perimeter, the transfer of moisture from the oceans to the inland regions was hampered. The regression of inland seas associated with global warming has led to the establishment of an arid (desert) climate in many zones, which further contributed to the increase in temperature. It was the way how the transition from the Ice Age to the Ice-Free era took place [9]. The duration of the Ice-Free era was about 200 million years. During this period, the evolution of life on Earth has come a long way, impossible in the temperature conditions of the Ice Age. And the first who left the ice captivity were turtles.

In **Figure 1**, three examples show the poses of turtles that they take during periods of basking—a special behavior aimed at heating. All the fragments show that the turtles expose the dorsal surface of the head to the rays of the sun. For orientation by the sun, the diencephalon of turtles has a light-sensitive pineal organ that reacts to sunlight [12] [13]. Its position on the dorsal side of the brain [12] indicates that it is the brain structures that require additional heating.

The connection between the processes of brain functioning and temperature was mentioned in the |Introduction| section due to the fact that the range of preferred temperatures in reptiles (28˚C - 32˚C [3]) coincides with the zone of increased spontaneous activity of brain neurons (27˚C - 29˚C [4]). In turn, the regulation of spontaneous activity is a determining factor for the formation of adaptive reactions [5] [6]. In the course of adaptive activity, the brain produces blocking the K^+ channels of neuronal membranes with the help of the M-cholinergic reaction [14]. Its manifestation lies in the ability of neurons to form, under the influence of acetylcholine, a high-frequency spike sequence from EPSPs passing through the dendrites [15], which leads to an increase in the level of spontaneous activity. Consequently: 1) there exist specialized cholinergic nuclei in the brain, from which neurons acetylcholine is released [16]; 2) the adaptive mechanism of the brain requires energy supply [17] [18] [19] because the M-cholinergic reaction is an energy-consuming process [20] and; 3) the impulse sequence formed by adaptive mechanism creates a nonspecific increase in excitation, without which an adaptive reaction of perception [6] or motor command to an external signal [5] would be impossible. Naturally, adaptive behavior is the more diverse, the wider the range of regulation of spontaneous activity.

The adaptive process proceeds in the same way in all temperature ranges characteristic of different groups of vertebrates and invertebrates, and differs only in the rate of the M-cholinergic reaction, which depends on the energy and temperature conditions in which it proceeds, and on the presence of an appropriate substrate (density of K^+ channels) on neuronal membranes.

In the temperature range of 24˚C - 37˚C, the M-cholinergic reaction increased stepwise in two temperature zones: 27˚C - 29˚C and 34˚C - 36˚C [21]. In the same temperature zones, the level of spontaneous activity and its variability in different neurons increases [21] [22] compared to lower temperatures. Consequently, at preferred temperatures in reptiles in the zone of 28˚C and above, along with an increase in the frequency and variety of spontaneous activity of neurons, the behavioral repertoire expands [23], and new environmental niches, inaccessible to fish and amphibians are settled [7] [8]. In this regard, the thermoregulatory behavior of turtles is aimed at achieving and maintaining a temperature in the region of 28˚C - 32˚C (**Figures 1-5**).

Turtles are the only owners of the shell among the inhabitants of Earth [7]. The presence of the shell in conditions of additional heating at very high (45˚C - 50˚C) temperatures allows it to be used for heat accumulation and its preservation for a long time—**Figure 4**. This method of thermoregulation is preferable to shuttle behavior (**Figure 5**) or constant stay in optimal temperature conditions due to the fact that daily temperature fluctuations on land are very significant. Some sea turtles living in the stationary conditions of the warm ocean do not have a horny shell; its upper part (carapace) is replaced by a soft leathery formation, which is not preserved in fossil materials [7]. This suggested that the shell is an attribute of the terrestrial origin of turtles, and its transformation in sea turtles is associated with a change in habitat conditions [7]. Of course, the shell can be a reliable defense against enemies, but the ability to maintain the acquired temperature for a long time (4 - 6 hours) gives grounds to assert that the main function of the shell is related to providing optimal temperature conditions for the brain to work during the transition to a new level of evolutionary development.

The absence of similar life forms that, following the turtles, would use horny integumentary structures to correct body temperature has a logical explanation—after the first wave of warming that occurred immediately after the Carboniferous-Permian glaciation, a second wave arose [24], which by the end of the Permian period led to the widespread increase in temperature on Earth to values close to and even exceeding 30˚C. The global nature of this process explains the mass extinction of numerous representatives of the Ice Age [24] [25]. This mass tragedy, the most global in the entire history of the development of life on Earth, was associated not only with the fact of another wave of warming, but also with an exactly temperature point that exceeded 30˚C. At this point at t = 27˚C - 29˚C there is an increase in the energy needs of the brain due to a temperature increase in the rate of the M-cholinergic reaction [21], which requires significant energy costs for its implementation [20]. Therefore, the energy costs that have arisen above 30˚C cannot be satisfied in the animals of the Ice Age due to the insufficient development of their circulatory and respiratory organs. The developed permanent hypoxic pathology of the brain led to disruption of the ion homeostasis of neurons and the inevitable death of a huge number of organisms [26] [27] the life cycle of which is associated with temperatures below 28˚C. Therefore, with accuracy to of several degrees, it can be confirmed that at the end of the Permian period, the global temperature on Earth had settled above 30˚C, which led to the flowering of reptiles, since the external temperature coincided with their preferred temperature. Consequently, the need for the shell as a source of heating disappeared, and only turtles preserved it [7] as a vestige of the transitional stage to the Ice-Free era. At the same time, the shell has not lost its significance and functions perfectly in the new Ice Age of our time (**Figure 3**).

In order for the shell to maintain a high temperature for a long time, it must be heated in very hot temperature conditions. Therefore, all of 6 turtles in the course of experiments if only ones, stopped for a long time in the temperature compartment of 45˚C - 50˚C (**Figure 3**). This temperature range exceeds the temperatures critical for survival [3]. Turtle may die at a temperature of 38.5˚C [3]. Phenomena that occur during hyperthermia: disruption of the structure of cell membranes, weakening of the function of ion pumps, loss of control over metabolism in the cell [2]—occur at temperatures exceeding the normal physiological range of existence. For turtles, the upper limit of tolerance is limited by the second zone of growth in the rate of the M-cholinergic reaction at t = 34˚C - 36˚C [21], which requires additional energy supply [20]. Exceeding this temperature zone for a long time is associated for turtles with consequences similar to the events that occurred with the glacial fauna at the end of the Permian period [23] [24]. That is why voluntary stay in the 45˚C - 50˚C zone is fraught with the development of hyperthermic pathology, from which turtles are protected on the one hand, by the low density of K^+ channels on neuronal membranes [8] [22] [28] and the special function of mitochondria to prevent intracellular Ca^{2+} overload [29], as well as adequate thermo-dependent behavior: by limited stay in zone of ultra-high temperatures (no longer than 1.5 hours), protection of the head from excessive heating and long-term (many hours) avoidance of repeated visits to the compartment with a temperature of 45˚C - 50˚C. This difficult struggle for temperature conditions to ensure adaptive perfection only began in the Permian period with the appearance of turtles, and the Mesozoic era was ahead [30].

The Mesozoic era, including the Triassic, Jurassic and Cretaceous periods, lasted about 160 million years and was characterized by a hot climate, which was 6˚C - 9˚C warmer than the modern one. Despite the natural zonality, the warming was so significant that the level of the world ocean was 100 m higher than the current one. The glacial polar caps have completely disappeared. Due to the tectonic shifts of the continents in the Jurassic, in the equatorial zone and in the middle latitudes, the arid climate was widespread. The content of CO_2 in the atmosphere was 4 times higher than today's values [30]. Aridization of the climate, high content of CO_2 in the atmosphere and volcanic emissions pushed the temperature on Earth to the second barrier for the M-cholinergic reaction of the brain: after all, only three, four degrees remained from the temperature of the end of the Permian period to 34˚C - 36˚C.

The mass dominance of reptiles, which arose from the beginning of the Mesozoic era, passed into a new stage: under changing temperature conditions, the range of preferred temperatures, also changed: its coincidence with the second zone of temperature increase in the rate of the M-cholinergic process provided significant advantages, since it made possible to have neurons, whose spontaneous impulsation was characterized by very high-frequency sequences for any adaptive behavior (Figure 6(B)). For a new class of reptiles of the Mesozoic era, as well as for turtles of the early Permian period, it was required to maintain the temperature acquired as a result of heating. Therefore, their bodies were large, since the surface of a large object through which heat is exchanged with the environment is smaller in area compared to a small body. As a result, large bodies cool more slowly than small ones, and it is easier to maintain a high temperature. Perhaps the same function was performed by horny shields and outgrowths, as well as long necks, passing through which the blood had time to heat up in the sun before reaching the brain. It is the brain of ectotherms that requires increased heating (Figure 1), and the arid climate of the Ice-Free era ensured constant heat exchange. Today it is difficult to establish how else dinosaurs achieved the required temperature range of 34˚C - 36˚C, but this temperature barrier associated with the M-cholinergic response of the brain was successfully overcome. This is evidenced by 3 stages (see Table 1) of the growth of adaptive capabilities in cold-blooded animals, depending on the existing or achieved brain temperature.

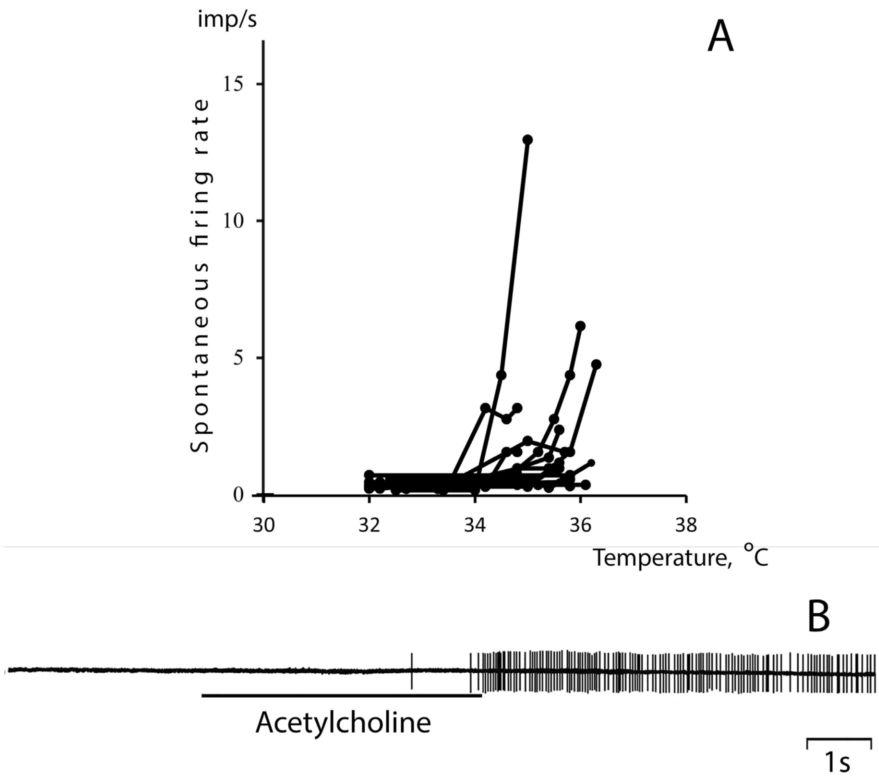

Figure 6. An increase in the rate of the M-cholinergic reaction in the temperature range of 34˚C - 36˚C. Presented the data of the article [6] obtained on neurons recorded in the slices of the sensorimotor cortex of guinea pigs. (A) Increase in spontaneous activity in 15 low-active neurons in the range of 34˚C - 36˚C. (B) The response of one of the neurons recorded in the range of 34˚C - 36˚C during microiontophoretic application of acetylcholine from a 2M solution of acetylcholine chloride (phoretic current 80 nA). The duration of the phoretic current is marked with a line under the record ofneuronal activity.

Table 1. Three stages of cold-blooded evolution.

	I stage	II stage	III stage
The rage of existing (I) or preferred (II, III) temperature	0˚C - 28˚C	28˚C - 32˚C	34˚C - 36˚C
Representatives among living groups	Tunicata, Acrania, Agnatha, Pisces, Amphibia	Reptilia	Dinosaurs
Types of environment	They are tied to aquatic territories	They colonized marine, fresh-water and terrestrial territories	They were the first who expand to the air

All three stages are associated with two temperature zones of an increase in the rate of the M-cholinergic reaction. The last stage fixes the temperature of 36˚C (**Figure 6**) as the base temperature for the evolutionary development of the warm-blooded.

5. Conclusions

The evolutionary transformation of the animal kingdom is closely connected with the adaptive mechanism of the brain. The adaptive mechanism is carried out when neurons form a high-frequency impulse sequence that accompanies the arrival of excitation from an external signal, but arises through a centrally determined process—the M-cholinergic mechanism of the brain [19] [31]. Any adaptive reaction is an active process, dependent on energy supply [17] [19] and temperature. Therefore, the development of the adaptive process was held back for a long time by the lack of sufficient oxygen in the Earth's atmosphere. It was only at the end of the Silurian period when the jawed fishes appeared, in the late Devonian they were joined by amphibians [11]. All of them belonged to the animals of the Ice Age—their life cycle proceeded in the temperature range of 0˚C - 28˚C [2]. The glacial era was interrupted by a rapid and extended warming of the climate, which led to the establishment of an Ice-Free era in the Permian period [9] and throughout the entire Mesozoic era [30]. It was the time when the development of adaptive process became thermo-dependent, occurred in two stages due to an increase in the rate of the M-cholinergic reaction in two temperature zones: 27˚C - 29˚C and 34˚C - 36˚C [21] [22]. Overcoming the first stage in the Permian led to the origin of reptiles with a preferred temperature of 28˚C - 32˚C. The temperature conditions of the Mesozoic era made it possible to overcome the second stage of the increase in the rate of the M-cholinergic reaction. This contributed to the appearance of a new class of reptiles with a preferred temperature above 34˚C that led to additional adaptive capabilities, in particular the ability to fly.

At all stages of the thermally dependent development of the adaptive mechanism, the need to maintain an adequate temperature of the brain (**Figures 1-5**) is the main condition for the existence of cold-blooded animals.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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