

Does Temperature Preference Change in Aging Flies?



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Introduction/Background

The importance of aging studies is evident given the ever increasing population of the elderly within the US. This has created a need to better understand the biological mechanisms of aging and to discover ways to prevent the detrimental effects of aging on the human body. However, it is very difficult to observe and study human's aging and therefore shorter-lived animals are used to study the mechanisms of aging. Fruit flies, *Drosophila melanogaster*, live an average 60-70 days; thus a day in the life of a fly are roughly equal to a year in the life of a human. This allow us to study many generations in different experimental conditions.

The Giebultowicz lab focuses on circadian rhythms and whether these rhythms become stronger or weaker as the individual ages. There are enough similarities between flies and mammals so that we can use flies as a model to understand biology of aging. We do this by comparing observations and making connections to our own human biochemical pathways, which regulate our life span. This is important because quantifying signs of aging in flies would allow us to know if circadian gene mutations are leading to increased pre-mature aging. Currently studies of aging rely on the shortened life span as a biomarker of overall health, while we seek to discover more concise quantitative means as an indication of aging. It was previously reported that circadian rhythm of temperature preferences exists in young flies (Kaneko et al. 2012, Goda et al. 2014). The purpose of this research was to determine if temperature preference patterns are altered in old flies and, if so, whether these changes could be used as a reliable and efficient biomarker of aging. To address these questions, we investigated temperature preference in young and old flies at two different times of day.

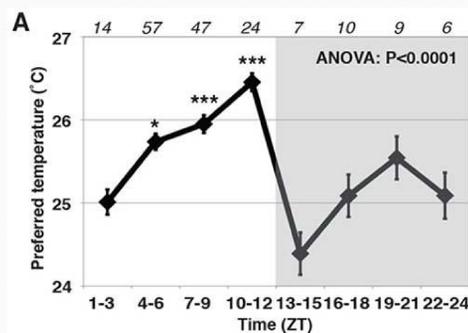
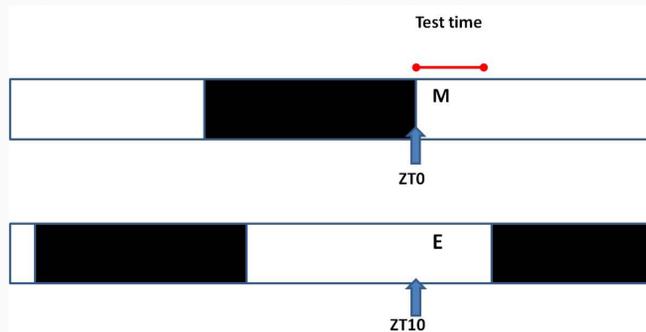


Figure 1. Previously reported pattern of a circadian rhythm in temperature preference observed in young flies (Kaneko et al. 2012).

Methods

Flies used in experiments were of the Canton Special (CS) genotype. The stocks were kept in 12h light: 12h dark (LD) cycles at 25°C. To measure simultaneously thermal preference of flies that were either in the morning phase or the evening phase of the circadian cycle, we kept half of the experimental flies in the incubators with reversed LD cycle as shown in Fig. 2. Flies used were of two age groups: young (Y) were between 5 and 20 days and old (O) were between 21 and 35 days old.

Figure 2. Experimental setup to measure simultaneously temporal preference of flies that were in the morning or evening phase or circadian cycle. Black bars represent 12 h of darkness, white bars represent 12 h of light. Flies were tested together for 30 minutes for each experimental trial during the 2 hour block starting at lights-on morning's Zeitgeber time (ZT) 0 and lights-off evening's ZT)10.



To measure temperature preference, we employed a simple and reliable method of creating an environment with a uniform temperature gradient. We used a rectangular 20 cm x 50 cm Aluminum plate of 6 mm. Each shorter edge of the plate was inserted into a Styrofoam box through a horizontal slit cut in the middle one of the box's vertical wall. Each slit was sealed with silicone caulk, and boxes were filled with warm or cold water. If the water temperature in one container is t_h , and in another one it is t_c , it will create a temperature gradient along the plate. Thermocouple probes were inserted into holes drilled in the aluminum such that the distance between the thermocouple probes was $D_{AB} = 31$ cm. In Fig. 3, the locations of probes are shown as the A and B points. Each probe was connected to an electronic meter (boxes th1 and th2 in Fig. 3) with a digital readout of the probe temperature in centigrade, with 0.1 °C precision. If the temperature at A is t_A , and at B is t_B , then the temperature gradient – i.e., the rate at which the temperature decreases per one centimeter of the plate length – is $grad(t) = (t_A - t_B)/D_{AB}$. Hence, the temperature at any point located between A and B, at a distance x to the right from A, can be obtained from a simple formula:

$$t(x) = t_A - x \frac{t_A - t_B}{D_{AB}} = t_A - x \nabla t$$

This equation was used to calculate temperature chosen by each fly in a given experimental group. After 30 min in temperature gradient, the entire plate containing flies in 4 channels was photographed (Figure 4), and the image was converted to that of flies set as "particles" which were assigned values for xy coordinates. The temperature preference was calculated from the position of each particle by using a computer program. At the end for each trial, we documented temperature ranges and determined each fly's temperature location along the plate based on their distance along the length of gradient. It was with these values that we analyzed the average temperature preference of each of the experiments as a function of fly's age and time of day. The data for young flies were compared to what was previously reported (Kaneko et al. 2012, Goda et al. 2014) to establish whether our apparatus was producing similar results.

References

- Goda, T., Leslie, J.R., Hamada, F.N. Design and Analysis of Temperature Preference Behavior and its Circadian Rhythm in *Drosophila*. 2014. Journal of Visual Experiments 83: 51097.
- Kaneko, H., L. Head, J. Ling, X.Tang, Y.Liu, P. Hardin, P. Emery, and F. Hamada. 2012. Circadian Rhythm of Temperature Preference and Its Neural Control in *Drosophila*. Current Biology 22: 1851-1857.
- Monk, T., D. Buysse, C. Reynolds, D. Kupfer, and P. Houck. 1995. Circadian temperature rhythms of older people. Experimental Gerontology 30: 455-474.

Figure 3. Schematic drawing of the temperature gradient apparatus used in the experiment. Flies were placed on the Aluminum plate under the plexiglass cover (C).

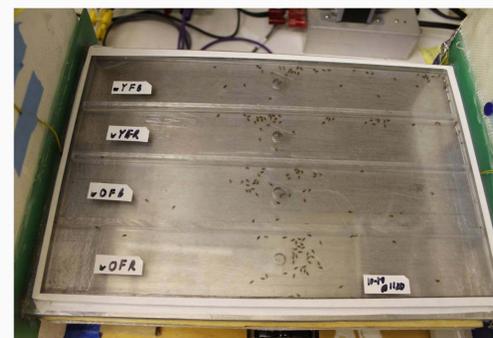
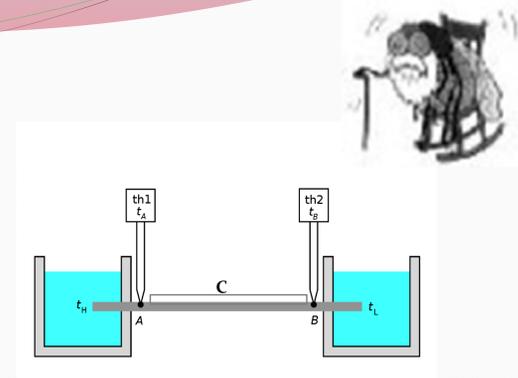


Figure 4. Photographic image of experimental flies within their separate channels after 30 minutes under the conditions of the temperature gradient.

Results

We tested temperature preference separately in CS females and males. Results obtained in females were not consistent between bio-repeats, probably due to technical variability. More reliable data were obtained in males, with two bio-repeats demonstrating preference for higher temperature in young males in the evening (Figure 5). This preference appeared to have been lost in old males (Figure 6).

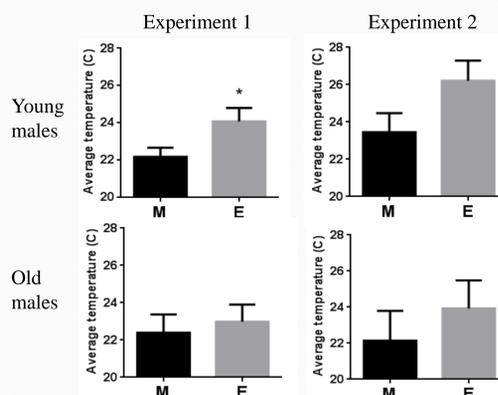
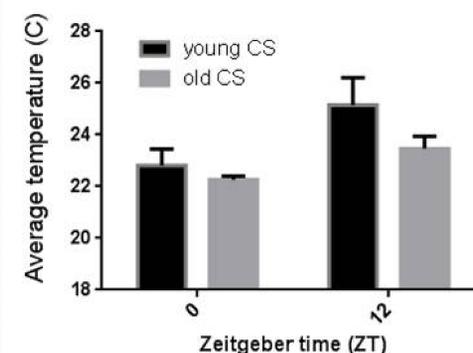


Figure 5. Bar graph comparing the average (\pm SEM) temperature preferences as function of time of day and age in two different experiments. In experiment 1, young flies show statistically significant ($p=0.034$) difference in preference with higher temperatures chosen in the evening. The same trend was observed in experiment 2, although difference was not statistically significant. There was no apparent tendency in older males to prefer higher temperature in the evening. M – morning, E – evening.

Figure 6. Summary graph comparing the average (\pm SEM) temperature preferences as function of time of day and age. Young flies show tendency to prefer higher temperatures in the evening as compared to the morning. Old flies appear to lose this tendency. (ZT0) – morning, (ZT)12 – evening.



Discussion and Conclusions

Our data confirmed that young flies have temperature preference rhythm and choose significantly higher temperature in the evening. The novel aspect of our study is the demonstration that older flies preferred to be in the colder temperature in the evening. There was no statistically significant difference between average temperature chosen by old flies in the morning or evening; therefore, we concluded that the rhythm of temperature preference is lost in old flies. Our data suggest that measuring temperature preference in the evening could serve as a reliable biomarker for indicative of aging. However, we encountered technical difficulties with our experimental set up. While there was a consistent pattern seen in a few trials, many trials were inconclusive because flies were crowding in the cold end of the plate, apparently immobilized by the low temperature of 15-16 °C that was part of the gradient. Further, the setting up and maintaining the apparatus was much more difficult than expected.

In conclusion, we discovered that the same evening preference for higher temperature that was observed in young flies did not occur in old flies. Interestingly, circadian body temperature rhythm is well known in young humans with the highest temperature observed in the evening. In a study that compared daily profile of body temperature in young and old humans, it was found that 80 year-old men had a tendency to display a leveling off of their body temperature rhythm as compared to humans aged 20 years (Monk et al. 1995). Remarkably, it appears that aging flies and humans show a similar trend toward external or internal lower evening temperature during aging.