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TEMPERATURE IMPACT ON PHOTOTACTIC BEHAVIOUR IN *DROSOPHILA MELANOGASTER*

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Declaration

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Content

Abstra	nct	4
Zusam	menfassung	4
1.	Introduction	5
2.	Materials and Methods	6
2.1.	Flies	6
2.1.1.	Fly Strains	6
2.1.2.	Fly Care and Treatment	6
2.2.	Experimental Setup and Procedure	8
2.2.1.	T-Maze	8
2.2.2.	T-Maze Phototaxis Assay	9
2.2.3.	Temperatures Phototaxis Assay in WTB Flies	10
2.2.4.	Considering Temperature as the Behavioural Changing Parameter	10
2.2.5.	The Behavioural Influence of the Genetic Background	10
2.3.	Statistical Analysis	10
3.	Results	11
3.1.	Light/Darkness Preference at Different Temperatures	11
3.1.1.	Determining Temperatures	11
3.1.2.	High Temperature Affects the Behaviour of Wing Clipped Flies	11
3.1.3.	Extending Decision Time	12
3.2.	Examining Temperature as the Behaviour Changing Parameter	14
3.3.	Temperature Impact on Light/Darkness Preference in CS Flies and <i>white</i>	19
3.3.1.	CS and White at Four Different Temperatures	20
4.	Discussion	24
Ackno	wledgements	26
Literat	ure	27
Figure	S	28

Abstract

The environmental and body temperature is important for the survival and development of flies. Humans can actively influence their body temperature among other things by wearing suitable clothes or drinking cold or hot beverages. The human body is also able to regulate the body temperature internally by perspiring or shivering. Flies don't have this ability, instead, they can only choose their environment according to their needs. The impact of temperature on the phototactic behaviour in flies was tested in this thesis. Therefore the behaviour of Wild-type Berlin (*WTB*) flies with and without wings were evaluated at different temperatures. Flies with clipped wings displayed a preference to choose the lighted area at the 37°C chamber, while they preferred darkness at lower temperatures. Flies with intact wings showed no behavioural change at any of the tested temperatures. Subsequently, transgenic flies were tested to proof temperature as the behavioural changing parameter, which was unsuccessful due to unknown issues. Six Canton S (CS) fly lines and the fly mutant *white* were also evaluated in the temperature phototaxis assay to test whether the genetic background has an influence on the flies' choice for either light or darkness and furthermore to verify potential differences between the strains. The most striking differences were found in flies with wings at the lowest temperature (17°C) and at the highest temperature (37°C).

Zusammenfassung

Die Umgebungs- und Körpertemperatur ist wichtig für das Überleben und die Entwicklung von Fliegen. Menschen können aktiv ihre Körpertemperatur unter anderem durch Tragen passender Kleidung oder Trinken kalter oder heißer Getränke beeinflussen. Der menschliche Körper ist auch in der Lage die Temperatur von innen heraus durch Schwitzen oder Frieren bzw. Zittern zu regulieren. Fliegen können das nicht. Sie müssen ihre Umgebung ihren Bedürfnissen entsprechend wählen. Der Einfluss von Temperatur auf das phototaktische Verhalten von Fliegen wurde in dieser Arbeit untersucht. Dazu wurde das Verhalten von Wild-typ Berlin (*WTB*) Fliegen mit und ohne Flügel bei verschiedenen Temperaturen ausgewertet. In der 37°C-Kammer zeigten Fliegen ohne Flügel eine Präferenz zum Licht, während sie bei niedrigeren Temperaturen die Dunkelheit bevorzugten. Fliegen mit beschnittenen Flügeln zeigten keine Verhaltensänderung bei keiner der untersuchten Temperaturen. Zusätzlich wurden transgene Fliegen getestet, um die Temperatur als den verhaltensverändernden Parameter zu beweisen, was wegen Probleme unbekannter Art erfolglos geblieben ist. Sechs Canton S (CS) Fliegenstämme und die Mutante *white* wurden ebenfalls in der Phototaxis Temperaturanalyse ausgewertet, um zu testen, ob der genetische Hintergrund die Wahl der Fliegen von Helligkeit oder Dunkelheit beeinflusst und um zusätzlich potentielle Unterschiede zwischen den Stämmen zu überprüfen. Die größten Unterschiede wurden bei Fliegen mit Flügeln bei der niedrigsten Temperatur (17°C) und bei der höchsten Temperatur (37°C) gefunden.

1. Introduction

It is important for all animals to regulate their body temperature and thus to sense temperature. In 1996 Benzer and Sayeed found that flies always prefer the cooler temperature, when they are exposed to a gradient from cold to hot. He also found out that the removal of the third antennal segment causes a loss of temperature sensing in flies, assuming that temperature sensing neurons are located in the antenna (Benzer and Sayeed, 1996). In 2008 Hamada showed that the ion channel TRPA1, which is located in the anterior cell neurons of the flies' head, is involved in sensing temperature (Hamada et al., 2008). Later GR28B(D) a gustatory receptor located in the hot cell neurons at the antenna/arista base was also found to be involved in temperature sensing (Ni et al., 2013), which proved Benzer's findings in the removal of the third antennal segment.

McEwan (1918) and Benzer (1967) showed that a fly's robust preference for light can be reduced by clipping its wings (McEwan, 1918; Benzer, 1967). Furthermore Gorostiza found that many interventions in the flying ability of a fly causes a change in light preference, showing that light preference is not just a simple response to a stimulus, it is more a decision-making process. (Gorostiza et al., in preparation). Unpublished data by Gorostiza showed that at 37°C *WTB* flies with intact wings become less positive and flies with clipped wings become almost neutral with a high variability.

In this thesis the impact of temperature on light preference was examined in flies with and without wings, basing on Gorostiza's unpublished data. Four temperatures for the temperature phototaxis assay were determined: 17°C, 25°C (room temperature), 32°C and 37°C. Once the light preference at all four temperatures was defined, it was evaluated if it was the temperature that influenced the light/darkness choice. Therefore the synaptic output was disrupted from the temperature sensing anterior cell neurons and the hot cell neurons by expressing *shi*^{TS}, which is a temperature sensitive mutation of the *shibire* gene taking *TrpA1*-GAL4 and *Gr28b.d*-GAL4 as drivers. This was unsuccessful due to unknown issues.

The genetic background can have an effect on the behaviour of flies (Colomb and Brembs 2014). To examine the influence of the genetic background on light preference at different temperatures several wild-type flies and the fly mutant *white* were investigated to show how it is involved in light and darkness preference at different temperatures.

The results showed that *WTB* flies with clipped wings are photopositive at the highest temperature, while they are negative at the lower temperatures. Flies with normal wings showed no behavioural change at any of the tested temperatures. Examining the genetic background revealed that the most striking differences between the strains appeared at the lowest (17°C) and the highest (37°C) temperature which shows that temperature affects the light preference differently even in normally identical fly lines.

2. Materials and Methods

2.1. Flies

2.1.1. Fly Strains

In this thesis experiments were conducted with different *Drosophila melanogaster* fly strains. In all experiments they were tested in groups of about 80 flies (40 with wings and 40 without), choosing them by chance in relation to gender.

UAS-*shibire*^{TS} (*shi*^{TS}), *Wild-type Berlin* (*WTB*), *CS*^{RE} (*Canton S Regensburg*) and *w*¹¹¹⁸ (*white*) were stocks from our lab in Regensburg. The *Gr28b.d*-GAL4 (58996) strain and the *TrpA1*-GAL4 (36362) strain were obtained from the Bloomington Drosophila Stock Center (NIH P400D018537).

Other five *Canton S* wildtype *Drosophila melanogaster* strains were: CS^{TZ} from Troy Zar's laboratory, CS^{TP} from Thomas Préat's laboratory, CS^{JC} derived from the CS^{TP} stock in 2007, CS^{BvS} from Bruno van Swinderen's laboratory and CS^{HS} from the lab of Henrike Scholz (Colomb and Brembs, 2014).

2.1.2. Fly Care and Treatment

2.1.2.1. Fly care

Flies were raised in vials holding standard cornmeal/molasses medium. A blot of living yeast mixed with a drop of water and a filter paper were placed in the middle of the food medium. By controlling the fly density per vial, approximately 35 flies, 20 females and 15 males, were put in a prepared vial for egg laying.

Flies were placed at a 25°C chamber with 60% humidity and a 12:12 hours light and dark cycle. They were transferred into new vials every day, except for those needed for collecting virgins and males for crossbreeds which were transferred into new vials every two to three days. Crossbred flies were placed at an 18°C chamber with a humidity of 60% and the identical light and dark cycle. Due to the lower temperature, which makes their development slower and the egg laying less, flies were transferred into new vials after three days and after another three days of egg laying they were discarded. This

procedure enables the larvae to liquefy the food medium during their different stages which indicates the ideal fly density per vial (Brembs, 2008).

2.1.2.2. The GAL4/UAS – System

To check whether a change in the behaviour might be due to the temperature sensed by the flies, the GAL4/UAS-system was expressed in specific temperature sensing neurons of the fly's head. GAL4 is a transcription factor from yeast with no endogenous target in Drosophila melanogaster. To express it in specific cells or tissues of a fly, it has to be under control of a driver gene, which needs to be naturally active in these cells or tissues. Once GAL4 is expressed it activates the transcription of a promotor that carries a GAL4 binding-site, the Upstream Activating Sequence (UAS). The UAS controls the expression of a gene of interest.

To control the expression of the gene of interest the GAL4 and the UAS are separated in two transgenic lines. The GAL4 is present in one line but cannot activate the gene of interest. In the other line the gene of interest remains silent because of the absence of the GAL4 (Brand and Perrimon, 1993). When crossing a fly holding the GAL4 with a fly carrying the UAS the GAL4/UAS-system is activated and the gene of interest is expressed in the specific cells or tissue.

2.1.2.3. *shi*^{TS} Expression to Inhibit Temperature Sensing

The GAL4/UAS-system was used to express a temperature sensitive mutation of the *Shibire* (*shi*) dynamine protein in temperature sensing neurons of the fly's head. *Shibire* is a membrane-bound protein that recycles synaptic vesicles. *Shibire*^{TS} is a dominant-negative mutation at the *shibire* locus and it blocks vesicle recycling at restrictive temperatures. If *shi*^{TS} is expressed in temperature sensing neurons of a fly, the neurons function is silenced. The drivers *Gr28b.d*-GAL4 and *TrpA1*-GAL4 were used to express UAS-*shi*^{TS}.

2.1.2.4. Fly Crosses

To assess if the preference between light and darkness depends on temperature, flies were crossed to *shi*^{TS} to silence temperature sensing neurons (for more details see table 1 below).

To generate *Gr28b.d>shi*^{Ts} flies *Gr28b.d-*GAL4 flies were crossed with UAS-*shi*^{TS} flies. Generating control flies *WTB* flies were crossed with flies carrying UAS-*shi*^{TS} and with flies carrying *Gr28b.d-*GAL4. *TrpA1>shi*^{TS} flies were generated crossing *TrpA1-*GAL4 flies with UAS-*shi*^{TS} flies. Control flies were generated by crossing *WTB* flies with UAS-*shi*^{TS} and with *TrpA1-*GAL4 flies. It was necessary to collect 20 virgins from one strain and 15 males from the other strain. The collected female flies were placed

at the 25°C chamber for at least three days to proof them to be virgin. Otherwise larvae would have been found in the vial which would have made the flies useless for crossbreeding.

It was necessary to collect only female flies from the *Gr28b.d*-GAL4 strain as the GAL4 system was located on the X-chromosome to assure that all the offspring have a functional GAL4/UAS-system. For egg laying the crossbred flies were placed at the permissive temperature for *shi*⁷⁵ (18°C).

Table 1: Crossbreeds.

CROSS PARTNER 1	CROSS PARTNER 2	RESULT	GROUP
WTB ♂/Ў	UAS- <i>shi™</i> ♂/¥	UAS-shi [™] / +	CONTROL GROUP
<i>Gr28b.d</i> -GAL4 ♀	WTB ♂	Gr28b.d-GAL4/ +	CONTROL GROUP
Gr28b.d-GAL4 ♀	UAS- <i>shi</i> ୮୨ ଟ	Gr28b.d>shi™	EXPERIMENTAL GROUP
WTB ♂/♀	UAS- <i>shi</i> ™ ರ′/¥	UAS-shi [™] / +	CONTROL GROUP
TrpA1-GAL4 రి/ర్గ	WTB ♂/Ў	TrpA1-GAL4/+	CONTROL GROUP
TrpA1-GAL4 రి/ర్గ	UAS-shi [™] ♂/¥	TrpA1>shi™	EXPERIMENTAL GROUP

2.1.2.5. Wing clipping

Since it is known that flies with clipped wings show a different behaviour to light from flies with intact wings (McEwan, 1918), it is investigated in this thesis if the phototactic behaviour of either flies with or without wings changes at different temperatures. Therefore wings of about 40 two to three days old flies were clipped choosing them by chance in relation to gender. They were anaesthetized under CO₂ for a short term. Meanwhile the wings were clipped to about 1/3 of their initial length. 40 wing clipped flies and 40 flies with intact wings were placed in a small vial with standard food medium. They recovered from the anaesthesia at 25°C (wild-type) or 18°C (crossbreeds) in a period 24 hours.

2.2. Experimental Setup and Procedure

2.2.1. T-Maze

To investigate the light preference of flies at different temperatures, a T-Maze apparatus was used (figure 1). In this apparatus a fly can choose between two conditions, light and darkness.

A T-Maze is made of an opaque PVC material and includes a mobile part with an elevator. The elevator is needed to transfer the flies to three different tubes which can be attached to the T-Maze. One is the entrance tube, an opaque tube with the length of 10 cm. This tube is used to adapt the flies to the

darkness and the paradigm. The other two tubes have a length of 20 cm, one of them is transparent made of acrylic and the other one is an opaque tube of PVC.



Figure 1: T-Maze. Apparatus used to assess the Temperature Phototaxis Assay; from http://lab.brembs.net/

2.2.2. T-Maze Phototaxis Assay

The T-Maze phototaxis assay was used to measure the light/darkness preference of flies. The temperature chamber was darkened during the experiment and a lamp was placed above the T-Maze to enable a homogenous light irradiation. A vial was placed in the climatic chamber to acclimatise it to the temperature. Once it had the same temperature as the chamber, the experimental group of flies, therefrom 40 with and 40 without wings, were placed in that vial and stayed there for 15 minutes for temperature adaption. Then they were gently pushed into the entrance tube of the T-Maze, where they had ten minutes to adapt to the darkness. After the adaption the elevator was pulled all the way up so that the flies could be pushed into the elevator. Then the movable part was shifted between the entrance tube and the other two tubes to prevent the flies from going back to the entrance tube to ensure that they participate in the experiment. They stayed in this position for 30 seconds to settle. To start the light/darkness choice the movable part was pushed all the way down, stimulating the flies to walk.

After every trial the number of the flies in the transparent tube (#FL), the opaque tube (#FD) and the elevator (#FE) were counted under CO₂. Also the total amount of flies (#FT) was determined. Then a Choice Index (CI) was calculated for both wing clipped flies and flies with intact wings using the belowmentioned formula:

$$CI = \frac{(\#FL \cdot (+1)) + (\#FD \cdot (-1)) + (\#FE \cdot (0))}{\#FT}$$

The highest Choice Index possible has a value of +1, the lowest a value of -1. Whereat a positive value means that the flies preferred the light, a negative value means the flies chose the darkness. A Choice Index of zero indicates no preference.

2.2.3. Temperatures Phototaxis Assay in WTB Flies

To test how wild-type flies behave at different temperatures according to light preference, the *WTB* (Wild-type Berlin) line's light/darkness preference was examined using a T-Maze. One group of flies was tested at one of the four determined temperatures: 17°C as the lowest temperature, 25°C as the preferred temperature, 32°C as a warmer temperature and 37°C as an increased temperature.

2.2.4. Considering Temperature as the Behavioural Changing Parameter

To investigate if the temperature might be the parameter that changes the phototactic behaviour in flies, the crossbreeds were tested at different temperatures (25°C, 32°C and 37°C). Therefore a T-Maze apparatus (figure 1) was used.

2.2.5. The Behavioural Influence of the Genetic Background

The influence the genetic background can have on a flies' behaviour was examined with different wildtype strains (*CS^{JC}*, *CS^{HS}*, *CS^{TP}*, *CS^{TZ}*, *CS^{BVS}*, *CS^{RE}*) and the fly mutant *white*. Each of the lines were tested in a T-Maze and the experimental setup and procedure remained the same as it was for the *WTB* line (see section 2.2.3 above).

2.3. Statistical Analysis

Statistical analysis was performed with the statistical program *InfoStat* (InfoStat Group, FCA, National University of Córdoba, Argentina). The normal distribution was tested with Shapiro-Wilks test. The homogeneity of variance was assessed with Levene's test. A p-value of p<0.05 was considered as statistically significant. In case of heterogeneity of variance and/or not normally distributed data, a non-parametric analysis was performed using the Kruskal Wallis test with pairwise comparisons as posthoc analysis (figure 2a, 3b, 8a/b, 9a/b, 10a/b, 11a/b). A one way ANOVA with Tukey as posthoc analysis was performed in case of homogeneity of variances and normally distributed data found in figure 2b and figure 3a. All experiments had an N of 8.

3. Results

3.1. Light/Darkness Preference at Different Temperatures

3.1.1. Determining Temperatures

Before starting the examination of the light preference at different temperatures, it was necessary to know if the flies can handle extreme temperatures.

To find a lowest temperature for the experiments, a small vial holding *Drosophila melanogaster WTB* flies was placed in a 4°C chamber. To have the same experimental conditions for all temperatures, flies needed to stay movable for at least 26 minutes which is the total amount of time a trial takes. At 4°C the flies got incapacitated after less than three minutes, which made it impossible to conduct experiments at this temperature.

The same was done in the 37°C chamber. Here the flies handled the temperature perfectly for more than 30 minutes. The other three temperatures used were 17°C as the lowest temperature, 25°C as the preferred temperature, and 32°C as an increased temperature. In those three temperature chambers no troubles appeared. Chambers with a temperature between 4°C and 17°C were not available in the laboratory.

3.1.2. High Temperature Affects the Behaviour of Wing Clipped Flies

WTB flies with and without wings were tested at the four above mentioned temperatures. The flies had 30 seconds for their light/darkness decision. This enables an investigation of a fast response to light after stimulating them to walk by pushing the elevator down as it was explained in section 2.2.2.

At all four temperatures the *WTB* flies with wings displayed a clear preference to light (figure 2a) showing no significant differences. In contrast flies with clipped wings showed a clear light avoidance at 17°C, 25°C and 32°C. Interestingly, flies with clipped wings obtained a positive CI at 37°C (figure 2b).



Figure 2: high temperature affects light preference in wing clipped flies. a, flies with wings preferred light at all four temperatures (CI at 17°C = 0.79, CI at 25°C = 0.56, CI at 32°C = 0.77, CI at 37°C = 0.63). Levene's test, p = 0.0298. Shapiro-Wilks, p = 0.0233. Kruskal Wallis, p = 0.1192. b, flies with clipped wings avoided the light at 17°C (CI = -0.17), 25°C (CI = -0.58) and 32°C (CI = -0.4), while they preferred the light at 37°C (CI = 0.5). Levene's test, p = 0.5591. Shapiro-Wilks, p = 0.0504. ANOVA p = <0.0001. Tukey, a common letter means no significant difference.

In unpublished experiments Gorostiza showed that flies with normal flying ability become less photopositive and flies with disruption in flying ability become almost neutral at 37°C. Assuming that the flies may need more time for the light/darkness decision, the time was extended from 30 seconds to three minutes to see if a light avoidance can be achieved when the flies have more time to decide and the stimulus mentioned above is no longer present.

3.1.3. Extending Decision Time

To determine whether the flies show a different light/darkness preference when giving them more time their slow response to light was examined at the four above mentioned temperatures.

Flies with wings showed a clear preference to light at 17°C, 25°C and 32°C, while they were highly variable at 37°C. However, at 32°C and 37°C they showed a reduction in the CI-Value (figure 3a).

Flies without wings remained photopositive at 37°C (figure 3b) although more flies chose the darkness compared to the previous results (figure 2b). At the other temperatures they still showed a clear preference to darkness (figure 3b).



Figure 3: Time extension in the temperature phototaxis assay. a, flies with normal wings showed a clear light preference at 17°C (CI = 0.4) and 25°C (CI = 0.53) but a reduction in light preference at the higher temperatures (CI at 32°C = 0.19, Cl at 37°C = 0.16). Levene's test, p = 0.2971. Shapiro-Wilks, p = 0.7508. ANOVA, p = 0.0092. Tukey, a common letter means no significant differences **b**, flies with clipped wings preferred the darkness at 17°C (CI = -0.48), 25°C (CI = -0.63) and 32°C (CI = -0.32), while they showed a preference to light at the highest temperature (CI at 37°C = 0.07). Levene's test, p = 0.0002. Shapiro Wilks, p = 0.0111. Kruskal-Wallis test p = 0.0009, a common letter means no significant difference.

The higher variability at 37°C led to the decision not to conduct further experiments examining their slow response to light.

3.2. Examining Temperature as the Behaviour Changing Parameter

In 2008 Hamada found that flies sense temperature via TRPA1, which is an ion channel located in the anterior cell neurons of the fly's brain (Hamada et al., 2008). Later Ni showed that flies can also sense temperature via GR28B(D), which is a gustatory receptor located in the hot cell neurons at the antenna/arista base (Ni et al., 2013).

To examine if the temperature caused the positive phototaxis in *WTB* wing clipped flies at 37°C, the temperature sensing neurons were silenced, using *Gr28b.d>shi*^{TS} and *TrpA1>shi*^{TS} flies. By expressing *shi*^{TS} under *Gr28b.d*-GAL4 and *TrpA1*-GAL4 drivers the neurons are silenced and therefore the flies are prevented from sensing temperature.

The flies were tested on three consecutive days at two different temperatures using the T-Maze apparatus. First at 25°C, second at one of the *shi*^{TS} restricted temperatures, 32°C or 37°C and a third time back at room temperature on the following day. As a change at restrictive 32°C of neither wing clipped flies nor flies with wings could be noticed in *WTB* flies, it was used as a control temperature where *shi*^{TS} is restricted. The hypothesis was that both *Gr28b.d>shi*^{TS} and *TrpA1>shi*^{TS} flies at restrictive 37°C show phototaxis like *WTB* flies at 25°C and that the high temperature was the cause of the behavioural change.

Experimental $Gr28b.d>shi^{TS}$ flies with intact wings were clearly photopositive at 25°C showing, however, small differences from control groups. Both control groups obtained a higher CI-value than the experimental group. Surprisingly the experimental flies showed no preference at 32°C (figure 4a), and the control group Gr28b.d/+ showed a lower light preference at 32°C than it was at 25°C, a behaviour *WTB* flies did not show at 32°C (figure 2a). The control group $shi^{TS}/+$ did not show notable differences at all three temperatures. Back at room temperature on the following day the experimental flies did not recover well from the heat treatment showing preference as well as avoidance, revealed through the high variability. Gr28b.d/+ recovered perfectly, obtaining a similar CI as at 25°C. However, in both control flies and experimental flies a high variability was obtained which makes comparisons between the groups and reliable statements difficult.

Wing clipped flies were clearly photonegative at all three temperatures, though showing notable differences in their behaviour (figure 4b). At 25°C experimental flies behaved nearly indistinguishably from control flies, while all groups became more photonegative at 32°C. Thereby control group $shi^{TS}/+$ obtained a more negative CI than the other two groups. After the heat the experimental group and the control *Gr28b.d/+* recovered from the heat, while $shi^{TS}/+$ remained more photonegative.

14



Figure 4: Examining temperature as the parameter changing the behaviour. a, flies showed light preference at 25°C, shi^{TS} /+ (CI = 0.57), Gr28b.d/+ (CI = 0.74), Gr28b.d> shi^{TS} (CI = 0.4), while the preference became less at 32°C, shi^{TS} /+ (CI = 0.39), Gr28b.d/+ (CI = 0.33), Gr28b.d> shi^{TS} (CI = -0.07). 24 hours after heat Gr28b.d> shi^{TS} became photopositive (CI = 0.27), shi^{TS} /+ (CI = 0.72), Gr28b.d/+ (CI = 0.78) recovered from heat **b**, flies with clipped wings showed a clear preference to darkness at 25°C shi^{TS} /+ (CI = -0.45), Gr28b.d/+ (CI = -0.31), Gr28b.d> shi^{TS} (CI = -0.43), 32° C shi^{TS} /+ (CI = -0.88), Gr28b.d/+ (CI = -0.51), Gr28b.d> shi^{TS} (CI = -0.66) and after the heat shock shi^{TS} /+ (CI = -0.81), Gr28b.d/+ (CI = -0.42), Gr28b.d> shi^{TS} (CI = -0.48)

Switching the temperature from 32°C to 37°C where the change in wing clipped flies was observed, another set of experiments was performed to try to proof that temperature is the behavioural changing parameter.

At 25°C experimental flies with wings showed a clear preference to light not differing much from control flies (figure 5a). Unexpectedly, at 32°C the experimental group obtained a negative CI, but was

highly variable, showing light preference as well as avoidance during experiments. The control groups showed almost no preference to light or darkness. 24 hours after the heat shock only the control groups seemed to have recovered, while the experimental group showed no preference.

However, all groups of wing clipped flies preferred the darkness at 25°C, but they obtained different CIs. While $Gr28b.d>shi^{TS}$ and Gr28b.d/+ were indistinguishably photonegative, $shi^{TS}/+$ obtained a lower



Figure 5: Examining temperature as the parameter changing the light preference at 37°C. a, flies with wings showed a distinct preference to light at 25°C $shi^{TS}/+$ (CI = 0.61), Gr28b.d/+ (CI = 0.79), $Gr28b.d>shi^{TS}$ (CI = 0.0.58), at 37°C flies preference became less $shi^{TS}/+$ (CI = 0.14) to no preference, Gr28b.d/+ (CI = 0.05) to avoidance $Gr28b.d>shi^{TS}$ (CI = -0.13), after 37°C heat $shi^{TS}/+$ (CI = 0.45) and Gr28b.d/+ (CI = 0.51) recovered, while $Gr28b.d>shi^{TS}$ (CI = 0.00) showed no preference. **b**, Flies with clipped wings preferred the darkness at 25°C, $shi^{TS}/+$ (CI = -0.47), Gr28b.d/+ (CI = -0.25), $Gr28b.d>shi^{TS}$ (CI = -0.58). At 37°C $shi^{TS}/+$ (CI = 0.03) showed no preference, Gr28b.d/+ (CI = 0.13) obtains a positive value and $Gr28b.d>shi^{TS}$ (CI = -0.21) became less negative. After heat all preferred the darkness $shi^{TS}/+$ (CI = -0.74), Gr28b.d/+ (CI = -0.49), $Gr28b.d>shi^{TS}$ (CI = -0.58).

negative CI but showed high variability. The experimental group were photonegative at restrictive 37°C (figure 5b), while both control groups showed no preference to light or darkness with high variability in the GAL4 control group. After the heat shock all groups became more negatively phototactic than they were at room temperature before the heat shock.

In sum, at both restricted temperatures, control temperature (32°C) and experimental temperature (37°C), a response was detected in the experimental group as well as in control groups. Therefore it was not possible to prove that the temperature changed the behaviour. In addition all groups obtained a high variability in both wing clipped flies and flies with intact wings, which makes it difficult to make reliable statements.

The same experiments were performed with the *TrpA1>shi*^{TS} flies and its associated controls, starting the examination with the restrictive 32°C set of experiments.

In all groups, the experimental group as well as control groups, flies with wings showed a clear preference to light at 25°C (figure 6a). At 32°C all groups obtained a lower CI than at room temperature before.*TrpA1>shi*^{TS} flies became more negative though, than the control groups. Furthermore,



on TrpA1-shi^{TS} flies. a, flies with wings showed a clear preference to light at $25^{\circ}C shi^{TS}/+ (CI = 0.77), TrpA1/+ (CI =$ 0.9), TrpA1>shi^{TS} (CI = 0.75). At 32°C flies showed less light preferences shi^{TS}/+ (CI = 0.44), TrpA1/+ (CI = 0.51), TrpA1>shi^{TS} (CI = 0.16). **b**, flies without wings showed light avoidance at 25°C shi^{TS}/+ (CI = -0.45), TrpA1/+ (CI = -0.17) as well as preference TrpA1>shi^{TS} (CI = 0.13). At 32° flies preferred the darkness, $shi^{TS}/+$ (CI = -0.74), TrpA1/+ (CI = -0.59), TrpA1>shi^{TS} (CI = -0.57). 24 hours after heat they also preferred the darkness $shi^{TS}/+$ (CI = -0.7), TrpA1/+ (CI = -0.6), $TrpA1>shi^{TS}$ (CI = -0.2).

TrpA1>shi^{TS} and *shi*^{TS}/+ flies were highly variable. After the heat shock flies in the experimental group and the control groups showed similar positive CI values, indicating a recovery from heat (figure 6a). Unexpectedly the experimental wing clipped flies obtained a positive CI at 25°C although the huge variability showed that in some experiments the flies preferred as well as avoided the light (figure 6b). Although the *TrpA1/+* control flies obtained a negative CI at room temperature, they were also highly variable like the experimental flies. Both groups became less variable and showed clear light avoidance at 32°C. Back at room temperature *TrpA1/+* control flies behaved almost the same as they did before at 32°C. The experimental flies were far less variable back at 25°C than they were before the heat shock. They even became photonegative, less photonegative, however, than at 32°C. *Shi*^{TS}/+ flies showed a clear preference to darkness at all three temperatures, although they became more photonegative at 32°C and at room temperature 24 hours after the 32°C heat.

At 32°C all groups showed a response to the restricted temperature, which the *WTB* flies did not. They were also highly variable especially in the GAL4 flies.

In another set of experiments the flies were also tested at the restricted 37°C where the behavioural change in wing clipped *WTB* flies was observed.

All groups of flies with wings showed a clear light preference at 25°C (figure 7a). At 37°C the experimental group and the *TrpA1/+* control group showed no preference to light or darkness, but they were highly variable. *Shi*^{TS}/+ showed a lower light preference at 37°C than at room temperature before. After the heat shock all groups showed a clear light preference and all seemed to have recovered from the treatment.

Unexpectedly experimental wing clipped flies became photopositive at 25°C, while *shi*^{TS}/+ obtained a negative CI value and *TrpA1*/+ showed no preference, but all groups were highly variable (figure 7b). At 37°C the experimental flies and the control *TrpA1*/+ obtained a negative CI, but remained highly variable. *Shi*^{TS}/+ showed almost no light preference. After the heat treatment the experimental flies and both control groups obtained a negative CI, whereby control flies obtained a lower CI and were less variable than experimental flies.

In total these results and the high variability obtained among all temperatures and in all genotypes neither proved nor disproved that temperature is the behaviour changing parameter. Although the experimental group of flies should not have sensed the temperature, a change in phototaxis was found in all flies.



Figure 7: The influence of 37°C on TrpA1-shi^{TS} flies. a, flies with wings showed a clear preference to light at 25°C shi^{TS}/+ (CI = 0.86), TrpA1/+ (CI = 0.9), *TrpA1>shi*^{TS} (CI = 0.9). At 37°C flies showed less light preference $shi^{TS}/+$ (CI = 0.3) to negative preference TrpA1/+ (CI = -0.13) or no preference $TrpA1>shi^{TS}$ (CI = 0.09). After heat they preferred the light shi^{TS}/+ (CI = 0.53). TrpA1/+ (CI = 0.7), $TrpA1>shi^{TS}$ (CI = 0.63) **b**, flies without wings at 25°C showed avoidance $shi^{TS}/+$ (CI = -0.35), no preference TrpA1/+ (CI = -0.08) and preference $TrpA1>shi^{TS}$ (CI = 0.34), at 32°C flies preferred the light $shi^{TS}/+$ (CI = 0.18) as well as avoided it, *TrpA1/+* (CI = -0.17), *TrpA1>shi*^{TS} (CI = -0.17)), after heat flies preferred the darkness, shi^{TS}/+ (CI = TrpA1/+ (CI = -0.52), -0.8), *TrpA1>shi*^{TS} (CI = -0.33).

3.3. Temperature Impact on Light/Darkness Preference in CS Flies and *white*

Since it is known that the genetic background can influence the behaviour of flies that are normally identical (Colomb and Brembs, 2014), it is suggested that this might have caused the results obtained in the previous experiments, as the genetic background of the *Gr28b.d*-GAL4, *TrpA1*-GAL4 and UAS-*shi*^{T5} flies was *white* and not *WTB*.

To test if the genetic background has an impact on the light preference at different temperatures six CS lines (see section 2.1.1) and the fly mutant *white* (w^{1118}) were tested.

3.3.1. CS and White at Four Different Temperatures

Not being able to proof temperature as the changing parameter, six different wild-type stocks and the mutant *white* were tested at the temperature phototaxis assay (see section 2.2.3).

Comparing all wild-type strains and the mutant at each temperature a significant difference in some of them was found.

At 17°C Canton S flies with wings showed high differences in their CI-values ranging from positive, like CS^{JC} , CS^{HS} , CS^{BvS} , CS^{RE} and CS^{TP} , to no preference, like the flies from Troy Zar's stock (CS^{TZ}) that showed a high variability. *White* obtained also a positive CI, but less than the photopositive CS lines (figure 8a). Wing clipped CS^{RE} and CS^{TP} flies showed a clear light preference while CS^{JC} , CS^{TZ} and *white* avoided the light. CS^{HS} and CS^{BvS} showed no preference, although CS^{BvS} was highly variable.



Figure 8: Canton lines at 17°C with and without wings. a, most CS flies with wings showed a clear preference to light (Cl $CS^{JC} = 0.78$, Cl $CS^{HS} = 0.74$, Cl $CS^{BvS} = 0.86$, Cl $CS^{RE} = 0.88$, Cl $CS^{TP} = 0.93$. CS^{TZ} (Cl = 0.02) and *white* (Cl = 0.26) showed less preference to light. Levene's test, p = 0.0053. Shapiro Wilks test, p = >0.0001. Kruskal-Wallis, p = <0.0001, common letter means no significant differences. **b**, flies without wings showed less light preference (Cl $CS^{HS} = 0.05$, Cl $CS^{BvS} = 0.01$, Cl $CS^{RE} = 0.45$, Cl $CS^{TP} = 0.59$), while some showed a preference to darkness (Cl $CS^{JC} = -0.08$, Cl $CS^{TZ} = -0.22$, Cl *white* = -0.18). Levene's test, p = 0.0199. Shapiro Wilks, p = >0.0001 Kruskal-Wallis, p = <0.0001.

Fly strains at 25°C with wings all showed a clear positive phototactic behaviour, with CS^{TZ} and white showing a slightly lower CI than the other fly strains (figure 9a). For the flies without wings CS^{HS} , CS^{RE} and CS^{TP} obtained a positive CI-value, while CS^{BVS} avoided the light and CS^{IC} , CS^{TZ} and white showed no preference (figure 9b). Among all wing clipped flies a high variability was obtained. All groups, except for CS^{RE} and CS^{TP} , showed light avoidance as well as preference during the experiments.



Figure 9: CS lines at 25°C. a, all CS flies with wings showed a clear preference to light (CI CS^{JC} = 0.89, CI CS^{HS} = 0.92, CI CS^{TZ} = 0.71, CI CS^{BVS} = 0.89, CI CS^{RE} = 0.96, CI CS^{TP} = 0.96, CI white = 0.69). Levene's test, p = 0.0108. Shapiro Wilks, p = >0.0001, Kruskal-Wallis, p = 0.0001, common letter means no significant difference. **b,** flies without wings showed less light preference (CI CS^{JC} = 0.07, CI CS^{HS} = 0.24, CI CS^{TZ} = 0.03, CI CS^{RE} = 0.63, CI CS^{TP} = 0.54, CI white = 0.01), while CS^{BVS} (CI = -0.11) showed a preference to darkness. Levene's test, p = 0.0367. Shapiro Wilks, p = 0.1317, Kruskal-Wallis, p = 0.0001, common letter means no significant difference.

At 32°C all fly strains with wings were clearly photopositive with no significant differences, although CS^{TZ} , CS^{BvS} and *white* obtained a slightly less positive CI than the other lines (figure 10a). In contrast, flies without wings showed positive CI-values like CS^{JC} , CS^{HS} , CS^{RE} , CS^{TP} and *white*. CS^{TZ} showed no preference. CS^{BvS} showed a negative CI-value (figure 10b). The variability for wing clipped flies especially for CS^{HS} was remarkably high.



Figure 10: CS lines at 32°C. a, all CS flies with wings showed a clear preference to light (CI $CS^{IC} = 0.86$, CI $CS^{HS} = 0.95$, CI $CS^{TZ} = 0.66$, CI $CS^{BvS} = 0.78$, CI $CS^{RE} = 0.92$, CI $CS^{TP} = 0.96$, CI *white* = 0.8). Levene's test, p = 0.1040. Shapiro Wilks, p = <0.0001. Kruskal Wallis, p = 0.0005, a common letter means no significant difference **b**, most CS lines without wings showed a light preference (CI $CS^{IC} = 0.25$, CI $CS^{HS} = 0.43$, CI $CS^{RE} = 0.44$, CI *white* = 0.27). CS^{BvS} (CI = -0.27) and CS^{TZ} (CI = -0.05) showed a preference to darkness. Levene's test, p = 0.0431. Shapiro Wilks, p = 0.1801, Kruskal-Wallis, p = 0.0010, a common letter means no significant difference.

At 37°C *CS^{IC}*, *CS^{HS}*, *CS^{TZ}*, *CS^{RE}* and *CS^{TP}* flies showed a clear light preference, while *white* preferred the darkness. *CS^{BvS}*, however, showed a light avoidance, in contrast to all the other Canton S lines (figure 11a). The same was observed in wing clipped CS flies. They also showed light preference, except for *CS^{BvS}* and *white*, which rather avoided the light. Contrary to all other lines *CS^{BvS}* and *CS^{RE}* flies were highly variable.

In sum differences in light preference were found between the fly lines among all temperatures. However, at 17°C CS^{TZ} and *White* with wings were significantly less photopositive than all the other lines. Furthermore at 37°C CS^{BvS} and *white* with wings even preferred the darkness to light.



Figure 11: CS lines at 37°C. a, most flies with wings showed a clear preference to light (CI $CS^{JC} = 0.83$, CI $CS^{HS} = 0.95$, CI $CS^{TZ} = 0.79$, CI $CS^{RE} = 0.81$ CI $CS^{TP} = 0.85$). CS^{BvS} (CI = -0.1) and *white* (CI = -0.25) showed a preference to darkness. Levene's test, p = 0.0348. Shapiro Wilks, p = >0.0001. Kruskal-Wallis, p = <0.0001, a common letter means no significant difference **b**, most flies without wings showed a light preference (CI $CS^{JC} = 0.82$, CI $CS^{HS} = 0.83$, CI $CS^{TZ} = 0.7$, CI $CS^{RE} = 0.41$, CI $CS^{TP} = 0.85$). CS^{BvS} (CI = -0.15) and *white* (CI = -0.33) showed a preference to darkness. Levene's test, p = 0.0160. Shapiro Wilks, p = >0.0001, Kruskal-Wallis, p = <0.0001, a common letter means no significant et al.

4. Discussion

Due to Gorostiza's unpublished findings, which showed that flies with disruption in flying ability become almost neutral to light and darkness and flies with normal flying ability become less photopositive in phototaxis at 37°C, the hypothesis was made that *WTB* flies with wings become less photopositive at higher temperatures, since light indicates unpleasant heat, and darkness suggests a cold place to hide. Wing clipped flies were expected to behave neutral to light and darkness at higher temperatures.

In contradiction to our hypothesis, WTB flies without wings became photopositive instead of neutral (figure 2b), while flies with wings showed a robust photopositive behaviour at all temperatures with no changes. But it is worth mentioning that flies with wings, which obtained more time for the light/darkness decision, and thus being able to settle and recover from the stress they experience through tapping and pushing in the T-Maze during the experiments, showed a lower light preference at 32°C and 37°C. In 1978 Markow examined different Drosophila species on their phototactic behaviour at low and high temperatures and low and high humidity and found that Drosophila melanogaster is more photopositive when temperature and humidity are high (Markow 1978). She and John supported earlier findings from Benzer, showing that agitated flies, which are exposed to stress, are photopositive (Benzer, 1967; Markow and John, 1976). Furthermore they showed that undisturbed flies in the photomazes rather prefer the darkness (Markow and John, 1976). Those findings were more or less reflected in experiments in this thesis. The WTB flies with wings showed a clear photopositive behaviour when they had 30 seconds after stimulating to walk at all four temperatures (figure 2a), while the flies were less photopositive after three minutes after the stimulus (figure 3a). Experiments by Head also revealed that flies preferred a higher temperature when exposed to direct light instead of darkness (Head et al., 2015) which is widely equivalent to what was found in the experiments with flies becoming more photopositive at higher temperatures.

In these experiments as well as Markow's a higher activity of the flies at higher temperatures was observed. Therefore, it would be interesting to conduct experiments in Buridan's paradigm (for more detail see http://lab.brembs.net/) to measure the impact of temperature on activity and locomotion towards dark objects (indicating darkness) in *Drosophila melanogaster*. This could not be conducted in this thesis due to too short a time frame.

Inhibiting temperature sensing neurons by expressing UAS-*shi*^{TS} under *Gr28b.d*-GAL4 and *TrpA1*-GAL4, it was tried to proof temperature as the behaviour changing parameter. It was hypothesized that transgenic flies at 37°C behave as wild-type flies at 25°C, as *shi*^{TS} is restricted at higher temperatures. It was not possible to confirm our suggestion neither with the *TrpA1*-*shi*^{TS} nor with the *Gr28b.d*-*shi*^{TS} flies. All transgenic flies showed a change in phototactic behaviour from 25°C to both higher

temperatures, control temperature (32°C) as well as experimental temperature (37°C). In addition a high variability was obtained between most experiments, which might be due to the influence of other environmental impacts besides the temperature. To prove temperature as the behaviour changing parameter these experiments need to be repeated.

As Colomb and Brembs showed that normally identical wild-type flies show differences in walking behaviour they suggest that the founder effect is the major cause for this phenomenon (Colomb and Brembs, 2014), it was thought that the genetic background of the transgenic flies, which was *white*, might have had an impact on the peculiar results in the experiments with the transgenic flies.

Six different Canton S flies and the mutant *white* (w^{1118}) were tested on their phototaxis at the four temperatures, and found the most striking differences between the lines at the coldest (17°C) and the hottest (37°C) temperature. At 17°C CS^{TZ} flies and *white* both with wings showed a significantly lower light preference than the other fly lines. *White* was positive, however, while CS^{TZ} showed no light/darkness preference. Furthermore significant differences were found at 37°C in flies with intact wings. CS^{BvS} and *white* both avoided the light, while the other strains were clearly photopositive. With these results Colomb and Brembs findings can be supported, as CS^{TZ} and CS^{BvS} showed significantly different phototactic behaviour to the other wild-type strains. Additionally this might show that the genetic background may have been a parameter in the temperature proving experiments, which lead to these peculiar results.

The variability was consistently high within almost all experiments. A reason may have been that the temperature chambers have different tolerances in air conditioning. Flies, which are highly sensitive for environmental changes, could have sensed those deviation from the normal state. This could also have caused the peculiar results of the transgenic flies and the sudden light preference of wing clipped flies at 25°C observed in figure 6b and figure 7b. In addition troubles appeared with the acrylic tube of the T-Maze, which broke and had to be glued several times. The flies might have smelled the glue especially at the increased temperatures where it might have emitted vapours. It needs to be mentioned, though, that peculiar results also appeared with the intact tubes. A building site in the lab, causing a lot of noise, might have stressed the flies during the trials. Repeating the experiments with the transgenic flies when the building site is gone might help to find out if the temperature caused the change or if another environmental impact influenced the choice of the flies.

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Literature

Benzer, Seymour (1967): Behavioral mutants of Drosophila isolated by countercurrent distribution. *Proc Natl Acad Sci USA* 58, 1112 - 9.

Benzer, Seymour; Sayeed, Omer (1996): Behavioural genetics of thermosensation and hygrosensation on Drosophila. *Proc Natl Acad Sci USA* 93, 6079 - 6084.

Brand, Andrea H.; Perrimon, Norbert (1993): Targeted gene expression as a means of altering cell fates and generatingdominant phenotypes.*The company of Biologists Great Britain* 118, 401 - 415.

Brembs, Bjoern (2008): Operant learning of Drosophila at the torque meter. *Journal of visualized experiments: JoVE* 16, 731.

Colomb, Julien; Brembs, Björn (2014): Sub-strains of Drosophila Canton-S differ markedly in their locomotor behavior. *F1000Research* 3, 176.

Gorostiza, E. Axel; Colomb, Julien; Brembs, Björn (in preparation): A value-based behavioural choice underlies phototaxis in Drosophila.

Hamada, Fumika N.; Rosenzweig, Mark; Kang, Kyeongjin; Pulver, Stefan R.; Ghezzi, Alfredo; Jegla, Timothy J.; Garrity, Paul A. (2008): An internal thermal sensor controlling temperature preference in Drosophila. *Nature* 454 (7201), 217 - 220.

Head, Lauren M.; Tang, Xin; Hayley, Sean E.; Goda, Tadahiro; Umezaki, Yujiro; Chang, Elaine C. et al. (2015): The influence of light on temperature preference in Drosophila. *Current Biology: CB* 25 (8), 1063 - 1068.

Markow, Ann Therese (1978): Phototactic Behavior of Drosophila Species at Different Temperatures The American Society of Naturalists 6, 884 - 892.

Markow, Therese A.; John, Merriam (1976): Phototactic and geotactic behavior of countercurrent defective mutants of *Drosophila melanogaster*. *Behav. Genet.* 7(6), 447 - 55.

McEwan, Robert Stanley (1918): The reactions to light and to gravity in Drosophila and its mutants. Journal of Experimental Zoology 25, 49 - 106.

Ni, Lina; Bronk, Peter; Chang, Elaine C.; Lowell, April M.; Flam, Juliette O.; Panzano, Vincent C. et al. (2013): A gustatory receptor paralogue controls rapid warmth avoidance in Drosophila. *Nature* 500, 580 – 584.

Figures

Figure 1: T-Maze	9
Figure 2: high temperature affects light preference in wing clipped flies	12
Figure 3: Time extension in the temperature phototaxis assay.	13
Figure 4: Examining temperature as the parameter changing the behaviour.	15
Figure 5: Examining temperature as the parameter changing the light preference at 37°C.	16
Figure 6: The influence of temperature on <i>TrpA1-shi^{TS}</i> flies.	17
Figure 7: The influence of 37°C on <i>TrpA1-shi</i> ^{TS} flies.	19
Figure 8: Canton lines at 17°C with and without wings	20
Figure 9: CS lines at 25°C.	21
Figure 10: CS lines at 32°C	22
Figure 11: CS lines at 37°C	23