Naive Gustatory Behavior of Wild-Type *Drosophila Melanogaster* Larvae in Response to Varying NaCl and Fructose Concentrations

Bachelor Thesis
Faculty for Biology and Preclinical Medicine
University of Regensburg
Institute for Zoology
Department of Neurogenetics

Supervisor: Prof. Dr. Björn Brembs
Submitted by Nils Henseling
Regensburg, August 2025

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List of Abbreviations

DO/DOG	Dorsal Organ/ Dorsal Organ Ganglion
Fru	Fructose
GR43a	Gustatory Receptor 43a
GRNs	Gustatory Receptor Neurons
IR76b	Ionotropic Receptor 76b
L3 Larvae	Third-Instar Larvae
M	Molar
NaCl	Sodium chloride
PI	Preference Index
PPK	Pickpocket
TO/TOG	Terminal Organ/ Terminal Organ Ganglion
VO/VOG	Ventral Organ/Ventral Organ Ganglion
WT	Wild-Type

Abstract

Understanding how animals respond to different tastants helps to uncover the sensory processes involved in making choices. To investigate this, this study tested how varying concentrations of fructose and NaCl affect naive gustatory behavior in *Drosophila melanogaster* larvae (WT CantonS). Additionally, it explored whether different lighting conditions and longer exposure times influence these responses. It has been shown that blue and red light did not affect the larvae's behavior towards different salt concentrations. Furthermore, a gradually decreasing aversive behavior with increasing salt concentrations was observed. sugar preference followed the expected inverted U-shape curve, with the strongest appetitive behavior at intermediate fructose concentrations and weaker responses at both low and high concentrations. Longer observation times led to clearly more distinct and stable behavioral responses, although this effect was primarily seen at higher concentrations. However, the project's results are only partially conclusive, since the findings from the salt avoidance experiment have not fully aligned with established literature. This highlights the need for further experiments to clarify the observed differences.

Zusammenfassung

Das Verständnis darüber, wie Tiere auf verschiedene Geschmäcker reagieren, hilft dabei die sensorischen Prozesse aufzudecken, die an Entscheidungsfindung beteiligt sind. Um dem nachzugehen, wurde in dieser Arbeit untersucht, wie unterschiedliche Konzentrationen von Fructose und NaCl das naive gustatorische Verhalten von *Drosophila melanogaster* Larven (WT CantonS) beeinflussen. Zudem wurde untersucht, ob es durch unterschiedliche Lichtverhältnisse und längere Expositionszeit beeinflusst wird. Es wurde gezeigt, dass blaues und rotes Licht keinen Einfluss auf das Verhalten der Larven gegenüber den verschiedenen Salzkonzentrationen hatte. Darüber hinaus wurde ein allmählich schwächer werdendes aversives Verhalten bei steigenden Salzkonzentrationen beobachtet. Die Präferenz für Zucker folgte der erwarteten umgekehrten U-förmigen Kurve: Die stärkste appetitive Reaktion zeigte sich bei mittleren Fructose Konzentrationen, während bei niedrigen und hohen Konzentrationen schwächere Reaktionen beobachtet wurden. Längere Beobachtungszeiten führten zu deutlich klareren und stabileren Verhaltensreaktionen, wobei dieser Effekt hauptsächlich bei höheren Konzentrationen auftrat. Wie auch immer, die beobachteten Ergebnisse sind jedoch nur teilweise schlüssig, da die Ergebnisse im „Salt Avoidance“ Experiment nicht vollständig mit bestehender Literatur übereinstimmen. Dies unterstreicht die Notwendigkeit weiterer Experimente, um die beobachteten Unterschiede zu klären.

1. Introduction

Whether humans rejecting a salty soup or larvae choosing between sugar and salt, the ability to judge the quality of food is a fundamental behavior across species (Yarmolinsky et al., 2009). This basic ability to distinguish between beneficial and harmful substances, has been shaped by evolution to assure that organisms increase their chance of survival (Breslin, 2013). *Drosophila melanogaster* is an established model organism for studying sensory based behavior. Its innate and reliable responses to basic taste stimuli like sweet, bitter, or salty make it especially suitable for experiments on naive gustatory behavior (Kim et al., 2016; Niewalda et al., 2008; Schipanski et al., 2008). To better understand the mechanisms underlying the choice between different stimuli, it is essential to examine how gustatory information is detected and processed in the nervous system of *Drosophila* larvae. The basic anatomical principles responsible for larvae detecting and processing gustatory information are already well established. Larvae possess three main external gustatory organs: the Terminal Organ (TO), the Dorsal Organ (DO), and the Ventral Organ (VO) (Gerber & Stocker, 2007). In addition, there are ganglia associated with these organs, the DOG (Dorsal Organ Ganglion), TOG (Terminal Organ Ganglion), and VOG (Ventral Organ Ganglion), which contain the cell bodies of the gustatory receptor neurons (GRNs) (Gerber & Stocker, 2007). The GRNs project via their neurites into the suboesophageal ganglion (SOG), the primary central processing area for taste in the larval brain (Maier et al., n.d.). While the DOG is primarily associated with olfactory sensing, the TOG and VOG serve mainly gustatory functions (Gerber & Stocker, 2007). Among the GRNs, one neuron expresses the receptor *Gr43a*, which is particularly sensitive to fructose (Miyamoto et al., 2012). Interestingly, *Gr43a* is also expressed in the larval brain, where it functions as an internal fructose sensor, potentially monitoring hemolymph sugar levels (Miyamoto et al., 2012). While in adult flies, certain gustatory receptor neurons expressing specific genes (*Gr5a* and *Gr64a*) are known to mediate sugar processing, it is still unclear whether these genes play a similar role in larvae, especially since reporter expression for these genes is often absent in larval stages (Colomb et al., 2007). In contrast to that, in adults, low NaCl concentrations (1mM-100mM) are sensed mainly by the ionotropic receptor *IR76b*, which promotes appetitive responses (Zhang et al., 2013). In Larvae, high salt concentrations, in contrast, are detected by members of the *Pickpocket* (PPK) family, specifically *PPK11* and *PPK19*, which trigger aversive behavior (Liu et al., 2003). Furthermore, it has been shown, that the *ppk11* gene in larvae, is essential for detecting low NaCl concentrations and is expressed in specific gustatory receptor neurons (GRNs) (Liu et al., 2003).

Additionally, a gene called *serrano*, which is being expressed in the TO also has an important role for detecting high NaCl concentrations (Alves et al., 2014). Moreover, *drosophila* larvae are known to show strong negative phototaxis, avoiding bright environments (Sawin-McCormack et al., 1995). Although there are no direct studies linking light exposure to gustatory naive behavior in larvae, it is assumable that light could indirectly influence gustatory choice by interfering with certain pathways.

The aim of this study was to find out how different concentrations of fructose and NaCl affect the naive gustatory behavior of WT *Drosophila melanogaster* larvae, and whether further factors like light condition or exposure time influence these responses.

Unexpectedly, salt avoidance behavior observed in larvae in the first experiment did not match previous findings by Niewalda et al. (2008), despite using the same WT strain (CantonS). This discrepancy raised the question whether methodological differences or possible defects in salt processing could explain the contrasting results. Therefore, experiments were appropriately modified to replicate established protocols. Furthermore, sugar preference behavior was tested as a control according to a paper, based on Schipanski et al. (2008), to test whether the larvae themselves are the reason for the discrepancies and to show if they accordingly respond to sugar unlike, they do to salt.

2. Materials and Methods

2.1 Fly Stock Maintenance and Rearing Conditions

The experiments were conducted using the *Drosophila melanogaster* Canton-S wild-type strain. Adult flies from a stock culture were first anesthetized using CO_2. Subsequently, 20 females and 10 males were selected and transferred into each of five separate glass vials containing a standard yeast-based medium. The flies were transferred to fresh vials daily to control larval age and ensure the consistent collection of stage L3 larvae. All cultures were maintained at 25 °C, 60% relative humidity, and a 12/12-hour light/dark cycle. After 6 days, the larvae had reached the third instar stage and were used for behavioral assays.

2.2 Preparation of Agarose Plates

Petri dishes (Sarstedt) were used for the behavioral assays. A visible line was drawn on the outside bottom of each dish to divide it into two halves. One half was labeled “P” (pure agarose), and the other half was labeled with the concentration of the respective substance. To prepare the plates, 1,5% Agarose Standard (Carl Roth) was weighed and dissolved by boiling in distilled water. The molten agarose solution was poured into the dishes and allowed to solidify at room temperature. After solidification, one half of the agarose was carefully removed along the drawn line using a spatula. The test substance (e.g., D(–)-Fructose >99.5% or NaCl >99%, Carl Roth) was weighed to achieve the desired concentration, mixed with freshly melted 1,5% agarose in water, and poured into the emptied half of the dish. Depending on the specific experiment, plates were either used immediately after preparation or stored overnight at room temperature prior to use.

2.3 Experimental Setup

Depending on the experiment, larvae were exposed to different lighting conditions or setups. The first two experiments were conducted under both red and blue light conditions: Blue light was provided by a light chamber, while LED strips and lamps were used to generate red light. White light experiments were performed under standard room lighting. Both in the salt avoidance experiment and in the sugar preference experiment, larval behavior was recorded over an extended period using a 4K-resolution camera mounted on a laboratory stand and connected to a laptop (**Figure 1**). The camera was positioned directly above the agarose plate, and the Petri dish lid was removed to ensure clear visibility.

Figure 1: Recording setup used in sugar preference and salt avoidance experiments: Visualization created using BioRender.com.

2.4 Behavioral Assays

2.4.1 General Larval Placement

For each trial, larvae were collected from one of five prepared glass vials. A small amount of larvae was transferred using a spatula into a water-filled Petri dish to wash off yeast and debris. Larvae found crawling on the dish edges were excluded to avoid including pre-pupating individuals. Exactly 30 larvae were collected using a small water droplet placed on a Petri dish lid, where surface tension kept them grouped. The larvae were then spread along the central dividing line of the agarose plates using a fine brush.

2.4.2 Red vs. Blue Light Experiments

An initial experiment tested whether blue and red light affect the naive gustatory behavior of wildtype CantonS larvae. Larvae were sequentially tested under red and blue light conditions, using three different substrate combinations: fructose(2M)/agarose, fructose(2M)/NaCl (1.5M), and NaCl (1.5M)/agarose. After a 3-minute test period, the number of larvae on each half of the Petri dish was counted. Larvae found on the dish lid were categorized as neutral and not counted for either side. This procedure was repeated for all trials.

A second experiment using the same lighting conditions assessed salt avoidance behavior. Larvae were tested with varying NaCl concentrations (0 M, 0.025 M, 0.5 M, 1.5 M, 2.5 M, 3.5 M), presented on one half of the Petri dish, with pure agarose on the other half. Larval distribution was determined after 3 minutes using the same counting procedure.

2.4.3 Salt Avoidance under White Light (Temporal Analysis)

After the red/blue light experiments, the same experiment was conducted under white light using the same NaCl concentrations and methods, except for the change in lighting conditions.

In a follow-up experiment based on a modified protocol from Niewalda et al. (2008), salt avoidance behavior was analyzed over time. Unlike the previous experiments, plates were prepared one day prior and stored at room temperature. Larvae were tested under white light for 15 minutes, with their behavior recorded using a 4K-camera mounted above the agarose plate. The Petri dish lid was removed to ensure visibility. Larvae leaving the plate were categorized as neutral. Larval positions were manually scored at 3, 5, 10, and 15-minute intervals.

2.4.4 Sugar Preference Assay under White Light

Finally, sugar preference was tested using various fructose concentrations (0.002M, 0.02M, 0.2 M, 2M, 4M). One half of the plate contained agarose mixed with fructose, while the other half contained pure agarose. This experiment followed the protocol from Schipanski et al. (2008), with slight modifications: 30 larvae per trial instead of 15 were used to improve data reliability. Experiments were conducted under white light and recorded using the same camera setup as in the temporal salt avoidance assay. Larval positions were counted after 90 seconds (as per the original protocol) and again after 180 seconds.

2.5 Evaluations

For data analysis, the raw data from the experiments was entered into an Excel spreadsheet containing a formula to calculate the preference indices of the individual test subjects. The formula is as follows:

$$PI\colon \frac{\#Stimulus - \#Pure}{\#Stimulus + \#Pure + \#Neutral}$$

In this formula, #Stimulus refers to all larvae that, depending on the experiment, positioned themselves on the side containing the tastant (either salt or sugar). #Pure includes all larvae that were located on the side containing only plain agarose without any tastants. #Neutral refers to larvae that either climbed onto the Petri dish lid or left the plate entirely. The result of subtracting #Pure from #Stimulus is then divided by the total number (#Stimulus + #Pure + #Neutral) of larvae to calculate the Preference Index (PI). Using this formula, the naive gustatory behavior of the larvae was quantified as a Preference Index, which ranges between -1 and +1. A positive PI indicates appetitive behavior, whereas a negative PI indicates aversive behavior.

2.6 Statistical Analysis

All data were analyzed using RStudio (version 4.5.0). Initial data inspection was performed using box plots. Normality was assessed for each group using the Shapiro-Wilk test. If the data were normally distributed, a two-sample t-test was used for comparing groups with each other, for not normally distributed data a wilcoxon rank sum test was performed. A one-sample sign test was performed for each experimental group to determine whether the median significantly differed from zero, indicating either appetitive or aversive behavior in the larvae. Levene’s test was used to assess homogeneity of variances between groups. Assuming homogeneity, a one-way ANOVA was conducted to compare all groups and test for an overall effect in the experiment. In order to account for multiple testing, a Bonferroni correction was applied to maintain the type I error rate at 5%. For a better overview, the results of the pairwise tests for plots with many groups were listed in tables in the attachment (**Table 1,2**). Significant results are indicated as follows: $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***.

3 Results

In the following section, the results of five experiments are presented. This bachelor thesis aimed to find out whether *Drosophila melanogaster* larvae of the Canton-S wildtype strain show changes in their naive gustatory behavior depending on salt and sugar concentrations, lighting conditions and also depending on how long larvae were exposed to the respective stimuli. The experiments are shown in the order they were conducted, starting with a gustatory control experiment, followed by salt and sugar choice assays.

3.1 Gustatory Control Experiment

Before conducting the actual experiments, it had to be assured that any effect by varying substrate concentrations appearing in the subsequent experiments is not attributed to different lighting conditions. Therefore, different substrate combinations were used to compare the larvae's naive behavior both under red and blue light. After 3 minutes the larvae were counted. Fructose (2M) / Pure Agarose: For both red and blue light, larvae show appetitive behavior towards sugar (**2A**; PI= 0,4 - 0,5). The same observation can be made in fructose (2M) / NaCl (1,5M), where larvae show an even stronger appetitive behavior towards fructose (**2C**; PI= 0,7 - 0,8), whereas in NaCl (1,5M) / Pure Agarose, aversive behavior can be observed towards NaCl (**2B**; PI = -0,4 - -0,6). However, statistical analysis proved no significant differences in naive behavior between red and blue light (**Figure 2**).

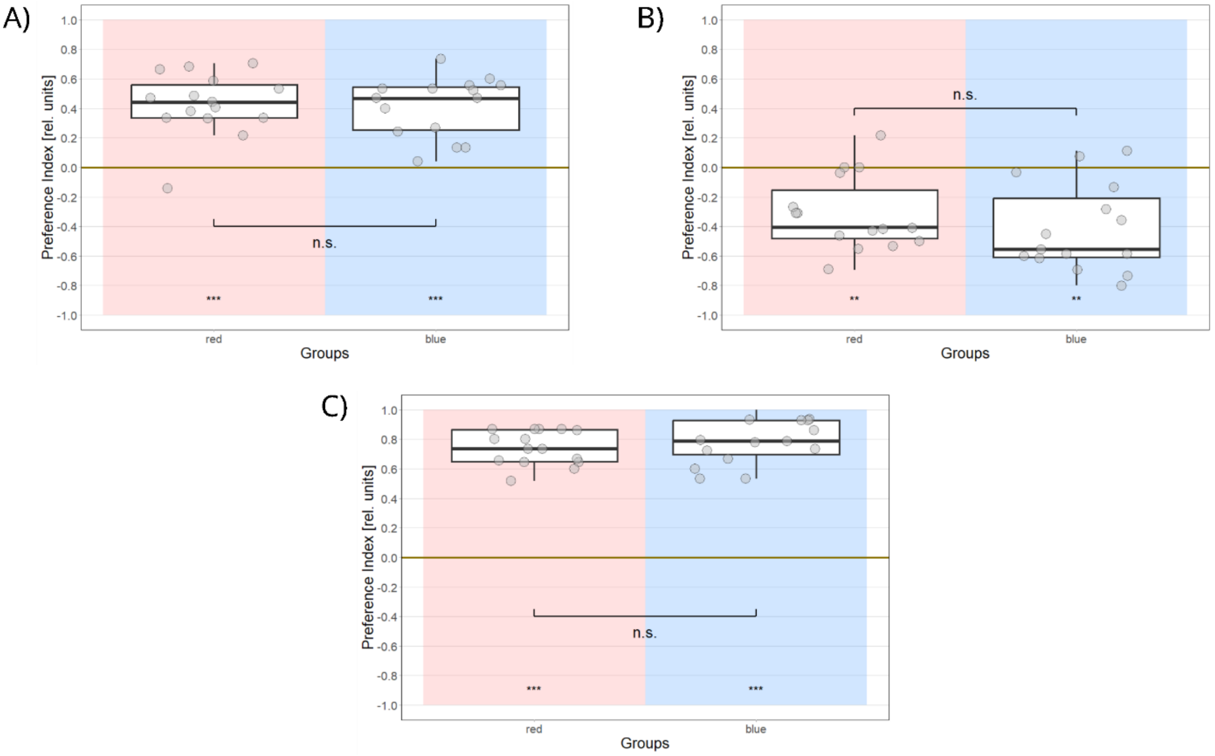

Figure 2: Gustatory control Experiment: *Performance Boxplots with Jitters; Each point represents the PI for one individual choice assay with 30 larvae; y-axis: Preference index [rel.units]; x-axis: light conditions. A) fructose(2M)/pure agarose); B) NaCl(1.5M) / pure agarose; C) fructose(2M) / NaCl(1.5M); N=15 for all groups; One-sample sign test for every group; Two sample t-tests for group comparison; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.*

3.2 Salt Avoidance under Blue and Red Light

Based on the results of the first experiment, which demonstrated that blue and red light have the same effect on naive gustatory behavior in CantonS larvae, in the following experiment was tested whether the same concludes for varying NaCl concentrations. Although the aversion from NaCl with the used concentration (1.5M) shows no significant difference between red and blue light (**2B**), the different NaCl concentrations could interact with the light factor resulting in varying behavior. To address this, the experiment was conducted and evaluated. For each NaCl Concentration a one-sample sign test was performed to check whether the median preference indices of the different groups significantly deviated from zero and additionally, a two-sample sign test was performed to compare the groups with each other. By comparing the test outcomes between red and blue light at each NaCl concentration, it was found that some concentrations (0 M, 0.025 M, 0.5 M, 3.5 M) showed similar trends, while others (1.5 M and 2.5 M) appeared to differ more strongly although none of these differences reached statistical significance (**Figure 3**).

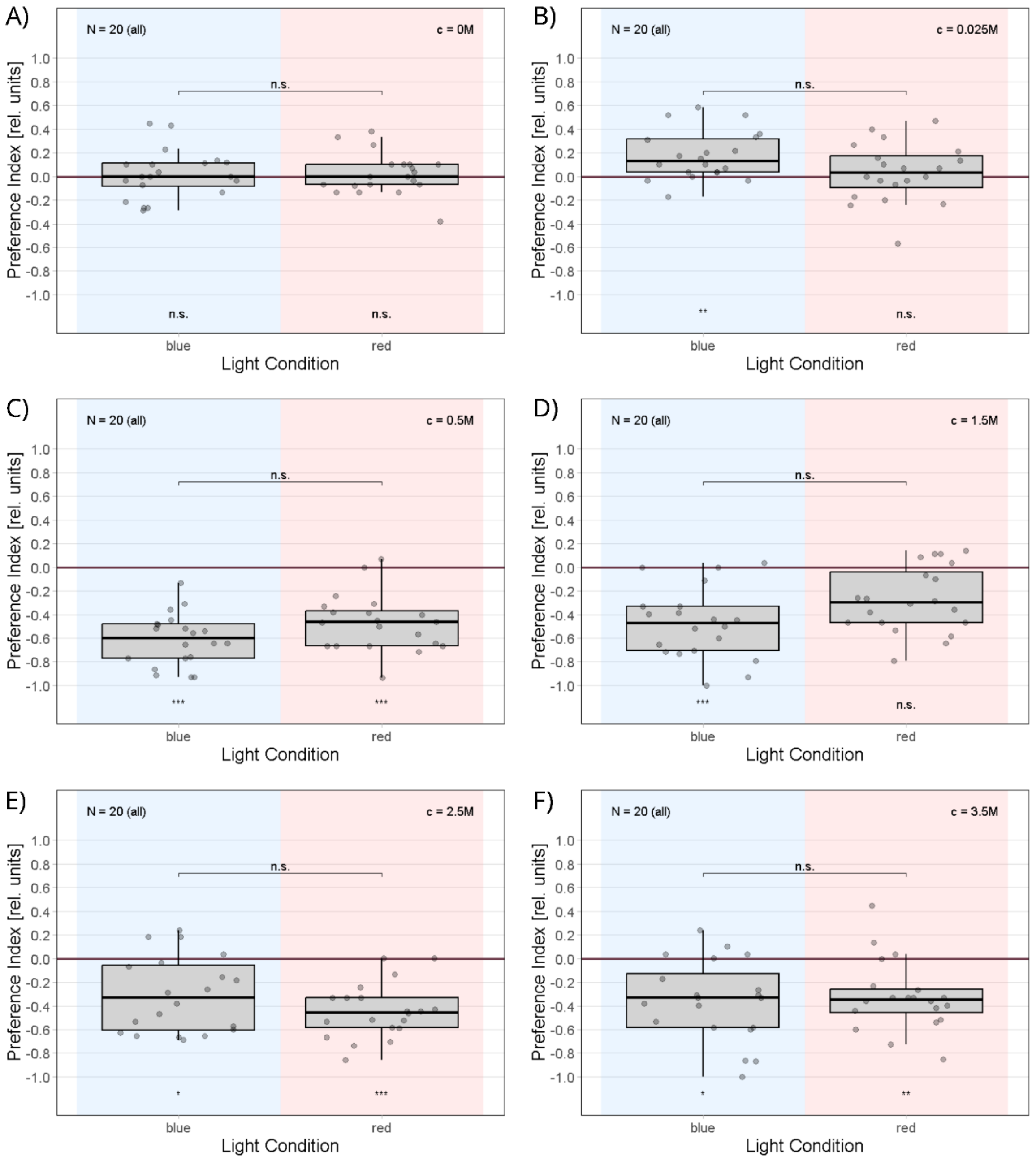

Figure 3: Salt Avoidance Comparison red- and blue light: Performace Boxplots with jitters; N=20 for all groups; y-axis: Preference Index [rel.units];x-axis: Light Condition; PI ranges from -1 to 1 with negative values indicating aversive behavior and positive values appetitve behavior; neighboring groups compared with two sample t-tests and one sample sign tests for every individual group; significance levels: p>0.05= n.s. ; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

The negative controls in both light conditions showed preference indices around zero (**3A**). At 0.025M NaCl, significant appetitive behavior was observed under blue light but not under red light, however, the group comparison was not significant (**3B**). At 0.5M NaCl, both red and blue light conditions showed strong aversive behavior, with a slightly stronger aversion under blue light (**3C**). At 1.5M NaCl, aversive behavior was significant under blue light but not under red light (**3D**). This pattern reversed at 2.5M NaCl, where larvae under red light showed a very significant aversion, while those under blue light showed a weaker but still significant aversion (**3E**). At 3.5M NaCl, both groups displayed significant aversive behavior (**3F**).

To visualize the overall trend of the larvae’s response to increasing salt concentrations under red- and blue light, two additional plots were generated. In both light conditions, the strongest avoidance was observed at a concentration of 0.5M NaCl, followed by a slow and gradual decline in avoidance behavior as salt concentration increased further.

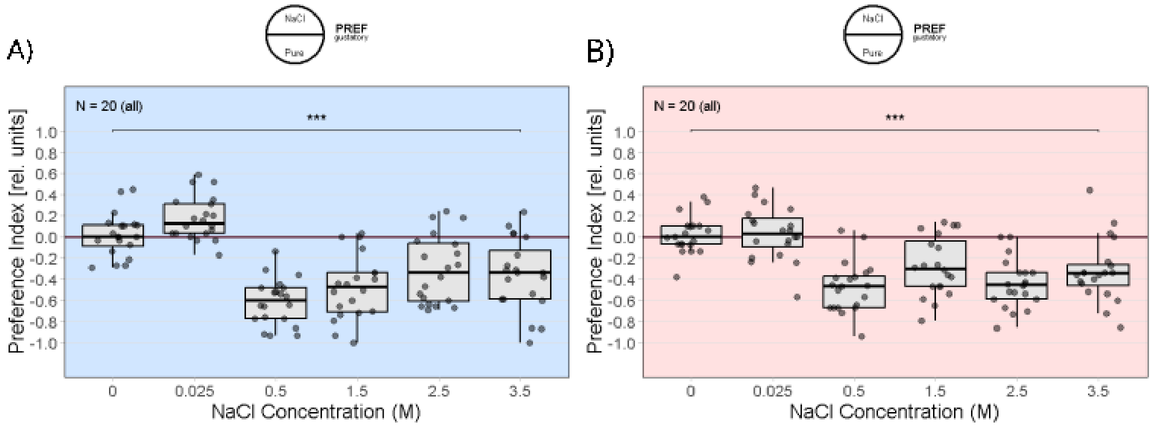

Figure 4: Salt Avoidance under blue (4A) and redlight 4B): Performance Boxplots with jitters; N=20 for all groups; y-axis: Preference Index [rel.units]; x-axis: NaCl Concentration [M]; PI ranges from -1 to 1 with negative values indicating aversive behavior and positive values appetitive behavior; ANOVA was used comparing all groups with each other; t-tests were used comparing neigboring groups with each other; significance levels: p>0.05= n.s. ; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

Since no significant differences were found between larvae tested under red and blue light conditions across various NaCl concentrations, the subsequent experiment was conducted under white light. Having tested a broad spectrum with red and blue light, proceeding with white light allowed us to standardize conditions and further investigate the larvae's gustatory behavior without competing effects from light.

3.3 Salt Avoidance under White Light

For this next experiment, the same setup was used as in the previous one, with the only difference being, that white light replaced the colored lighting. As shown below, there are similar results as in the recent experiment, showing a gradually decreasing aversive behavior starting at 0.5M NaCl all the way to 3.5M NaCl (**Figure 5**).

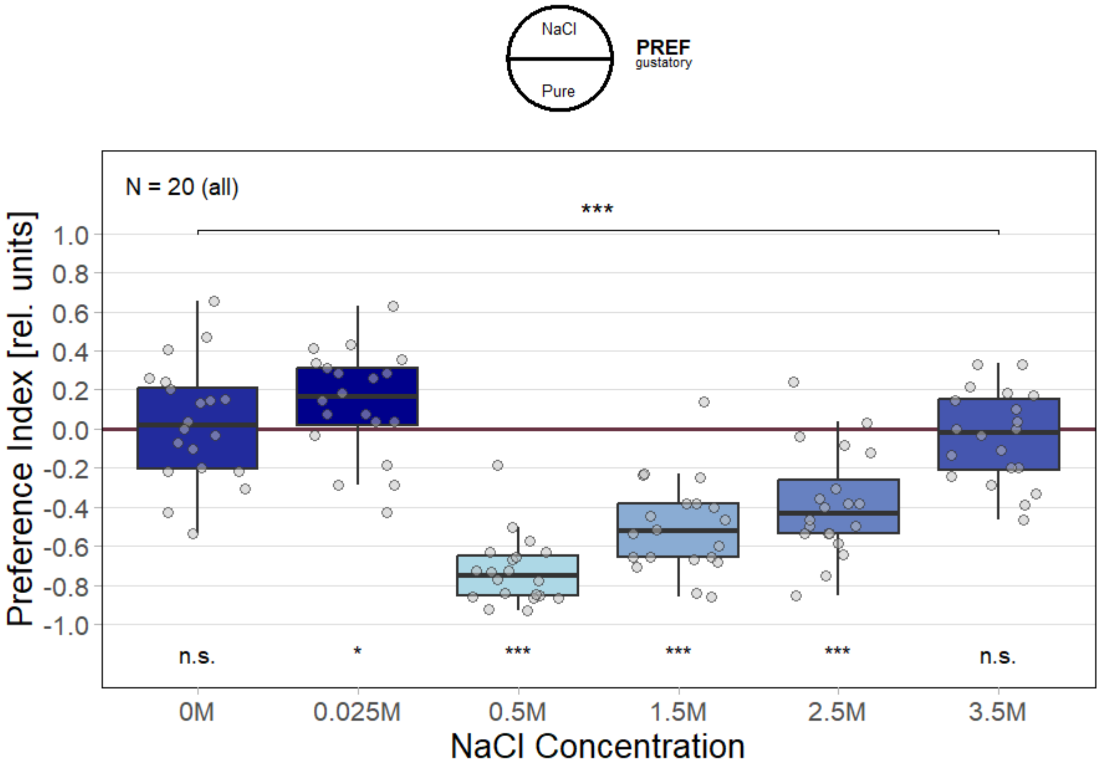

Figure 5: Salt Avoidance under white light: Performance Boxplot with jitters; N=20 for all Groups; y-axis: Preference index [rel.units]; x-axis: NaCl Concentration [M]; PI ranges from -1 to 1, with negative values indicating aversive behavior and positive values appetitive behavior; ; neighboring groups compared with two sample t-tests (Table 1) and one sample sign tests for every individual group; ANOVA was used comparing all groups with each other; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

With the negative control again being at a PI of zero, baseline neutrality is confirmed. At 0.025 M NaCl, larvae showed a weak appetitive response, consistent with the result observed under blue light. Strong aversion was observed at 0.5 M NaCl (PI = **–**0.75), with decreasing aversion at 1.5 M NaCl (PI= –0.5) and 2.5 M NaCl (PI= –0.4). At 3.5 M NaCl, the PI returned to approximately zero, indicating no clear preference. One-sample sign tests showed significant deviation from zero at all NaCl concentrations except 0 M and 3.5 M. Additionally, two-sample t-tests revealed significant differences between NaCl concentrations of 0.025 M and 0.5 M, 0.5 M and 1.5 M, and 2.5 M and 3.5 M (**Table 1**).

3.4 Salt Avoidance under White light (Temporal Analysis)

Given that the experimental design and NaCl concentrations were originally based on a paper by Niewalda et al. (2008), a comparison with their results revealed notable differences. While they reported, that behavior turns from appetitive to aversive as NaCl concentration is increased (Niewalda et al., 2008), in this experiment larvae showed a peak in aversion at 0.5 M, followed by a gradual decline at higher concentrations.. To investigate whether these discrepancies might be due to procedural differences, the experimental setup was modified as described in Materials and Methods (**2.5.3**).

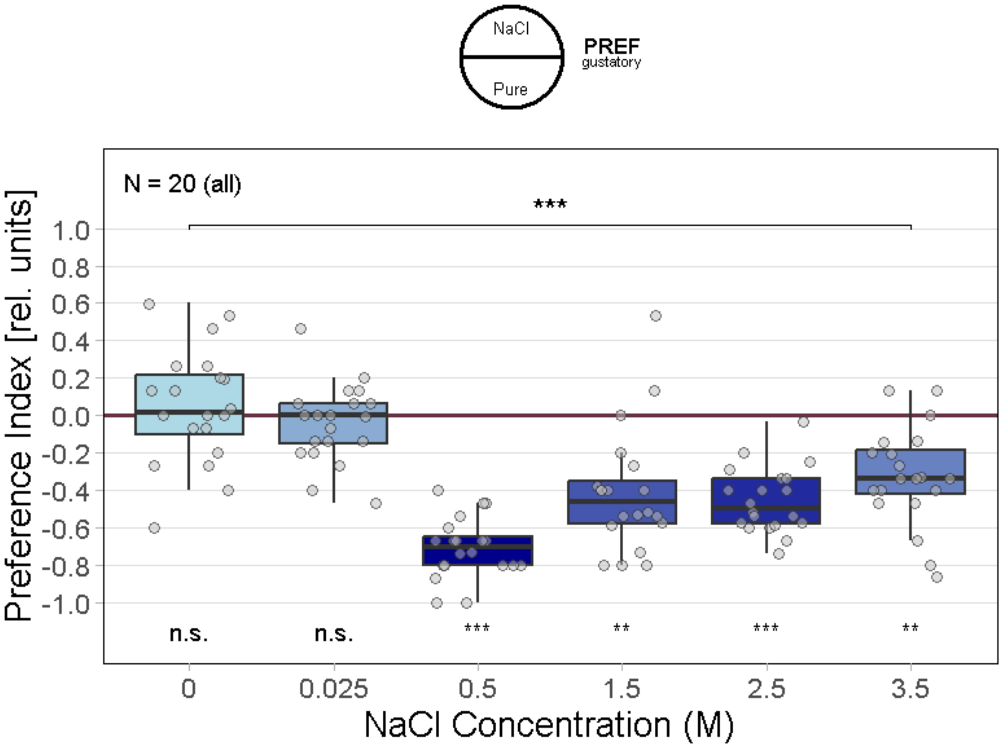

Figure 6:Salt Avoidance under white light after 15 minutes: Performance Boxplot with jitters; N=20 for all Groups; y-axis: Preference index [rel. units]; x-axis: NaCl Concentration [M]; PI ranges from -1 to 1, with negative values indicating aversive behavior and positive values appetitive behavior; ; neighboring groups compared with two sample t-tests (Table 1) and one sample sign tests for every individual group ANOVA was used comparing all groups with each other; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

The boxplot above displays the results after a 15-minute exposure period (**Figure 6**). Compared to the previous experiment (**Figure 5**), several differences occurred. For instance, at 0.025 M NaCl, larvae no longer exhibited a preference, whereas they previously showed weak appetitive behavior after 3 minutes. Furthermore, at 3.5 M NaCl, larvae displayed a clearly aversive behavior after 15 minutes, while no preference was observed at that concentration in the earlier experiment (**Figure 5**).

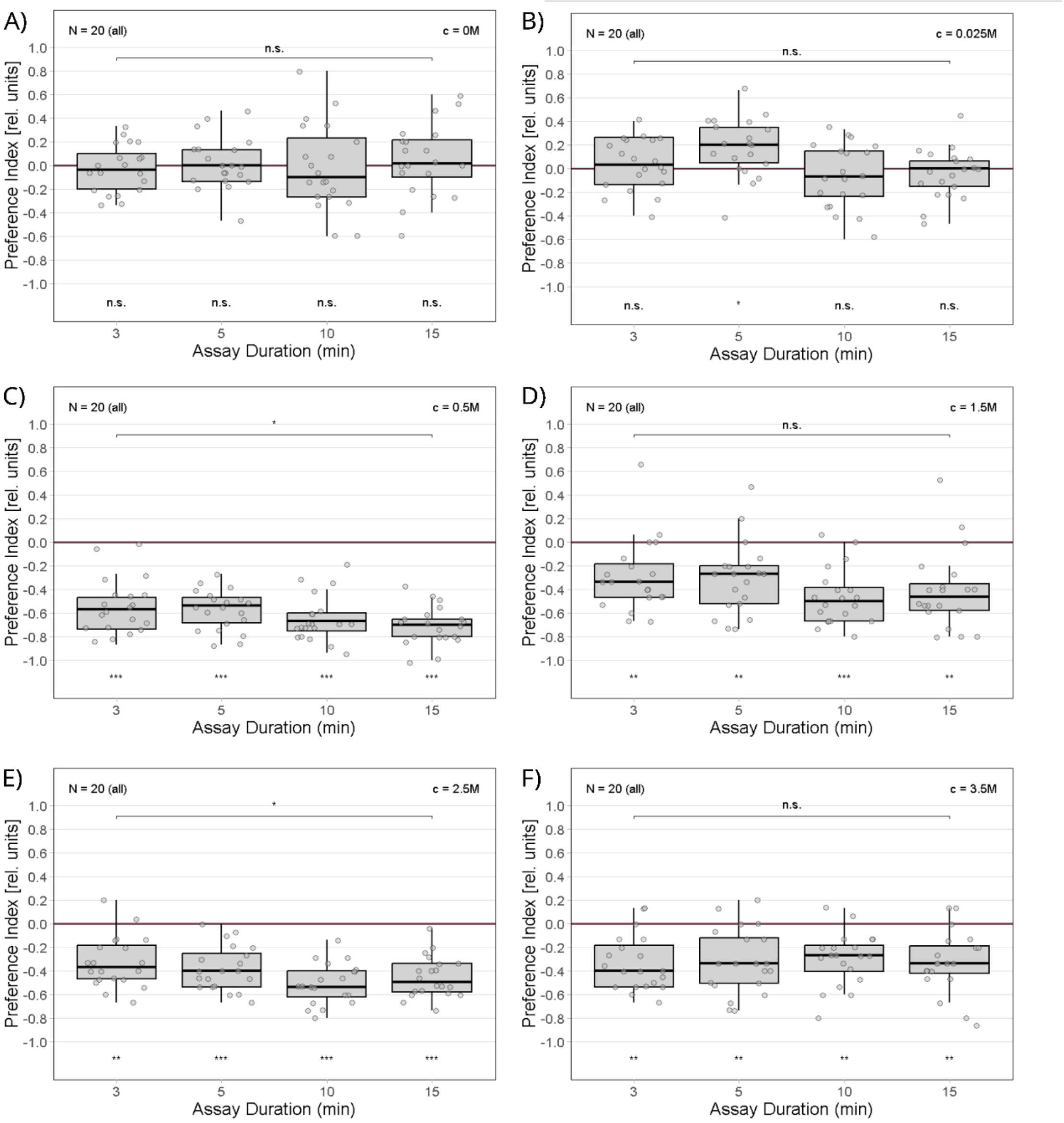

Figure 7: Salt Avoidance under white light (Temporal Analysis): A-F: Performance Boxplots with jitters; N=20 for all Groups; y-axis: Preference index [rel. units]; x-axis: Assay Duration (min); PI ranges from -1 to 1, with negative values indicating aversive behavior and positive values appetitive behavior; t-test for comparison of 3min and 15min; one sample sign tests were used for every individual group.; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

In order to analyze the temporal progression of larval behavior across different NaCl concentrations, all data were plotted as a function of time (**Figure 7**). Each NaCl concentration was evaluated at four time points: 3, 5, 10, and 15 minutes. Upon inspection of the boxplots, minimal differences in behavioral change over time could be observed. While gustatory preference remained relatively stable across all time points at NaCl concentrations of 0 M, 0.025 M, 1.5 M, and 3.5 M, larvae exposed to 0.5 M and 2.5 M NaCl exhibited a tendency toward increased aversive behavior at 15 minutes compared to 3 minutes.

Notably, this temporal shift was statistically confirmed by significant two-sample t-tests at these two concentrations (**Table 1**). However, even with the extended measurement period of 15 minutes, the results did not align with those reported by Niewalda et al. (2008).

3.5 Sugar Preference under White Light

In the final experiment, the focus was shifted to testing sugar preference instead of salt avoidance to determine whether the observed differences in data could be attributed to characteristics of the CantonS larvae. The experimental design was based on a paper by Schipanski et al. (2008), in which CantonS larvae were tested under white light with varying concentrations of sugar (fructose).

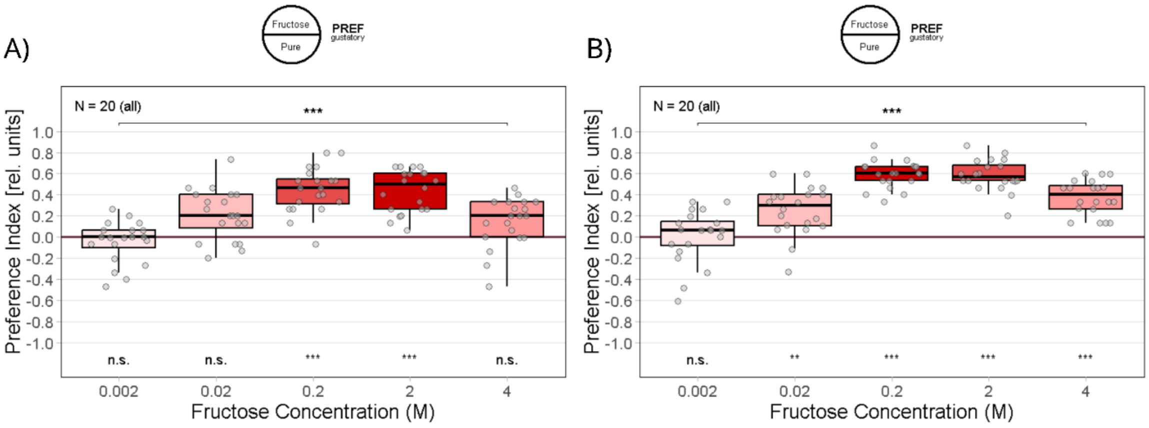

Figure 8: Sugar Preference under White light: Performance Boxplots with jitters; N=20 for all Groups; y-axis: Preference index [rel. units]; x-axis: Fructose Concentration (M); PI ranges from -1 to 1, with negative values indicating aversive behavior and positive values appetitive behavior; one sample sign tests against zero for every individual group; two-sample t-tests for comparing neighboring groups; ANOVA was used comparing all groups with each other; A) assay duration 90s; B) assay duration 180s; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

Larvae were observed for a total duration of 3 minutes, with larval positions recorded at two time points: after 90 seconds (**8A**) and again at 180 seconds (**8B**). In both resulting plots, the negative control shows a PI of zero. Across both time points, an increasing appetitive response was observed as fructose concentration increased, peaking between 0.2 M and 2 M fructose. Beyond 2 M, this trend reversed, with higher concentrations generating weaker appetitive responses. These findings are consistent with the results reported by Schipanski et al. (2008).

In addition, fructose concentrations were plotted against time points to evaluate, if larval behavior changed after an increased exposure time. At higher fructose concentrations (0.2 M, 2 M, and 4 M), larvae reached significantly increased preference indices at 180 seconds compared to 90 seconds, indicating a time-dependent boost of appetitive behavior (**9C, 9D, 9E**). Moreover, the reduced variability in PI values at 180 seconds suggests a more robust and consistent preference response following prolonged exposure.

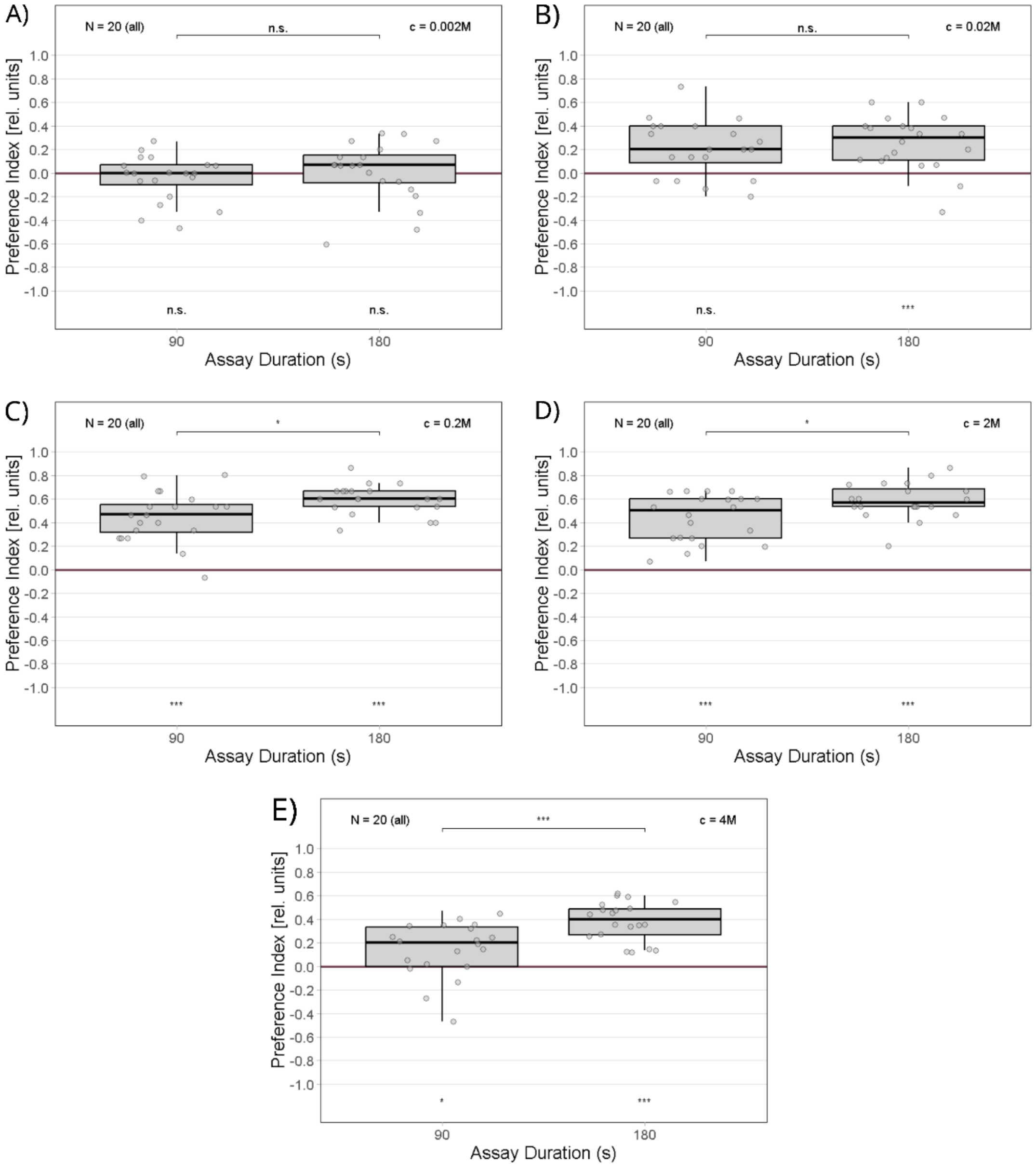

Figure 9: Sugar Preference under white light (temporal analysis): A) c =0.002M; B) c =0.02M C) c =0.2M D) c =2M E) c = 4M; Performance Boxplots with jitters; N=20 for all Groups; y-axis: Preference index [rel. units]; x-axis: Assay Duration (s); PI ranges from -1 to 1, with negative values indicating aversive behavior and positive values appetitive behavior; one sample sign tests against zero for every individual; two sample t-test for comparison of 90s and 180s; significance levels: p>0.05= n.s.; p<0.05 = *; p<0.01 = **; p<0.001 = ***.

4 Discussion

4.1 Control Experiment

Comparing larval naive gustatory behavior under red and blue light revealed no significant differences in naive gustatory responses across the respective substrate combinations. This indicates that the different lighting conditions did not affect the larvae's behavior. Therefore, the experiment can be considered successful and showed results as expected. However, an interesting side observation found, is that in the control experiment larvae displayed a stronger preference for sugar when the alternative option was salt, compared to when it was pure agarose (**2A, 2C**). This finding suggests that sugar preference might not be an absolute value but rather a context dependent one maybe enhanced by the aversive potential of the competing substance.

4.2 Salt Avoidance under Different Lighting Conditions

In all conducted salt avoidance experiments, a consistent trend was observed: The results in all experiments under the different lighting conditions were all reproducible and revealed the same pattern to increasing NaCl concentrations at all times. The strongest avoidance occurred at intermediate concentrations with the peak being at 0.5 M NaCl, while increasing NaCl concentrations were avoided less. This behavioral pattern stands in clear contrast to established findings, particularly to the paper by Niewalda et al. (2008). They reported an opposite relationship, where increasing NaCl concentrations, lead to stronger avoidance behavior in the larvae. A likely explanation for this difference may lay in the methodological differences between the studies. For instance, the salt avoidance experiment under red and blue light, was largely conducted with the help by my fellow lab partner Eva Schächtl. This helps to reduce the probability of human error or technical errors and supports the reliability of the findings. However, the experimental procedure differed from the methods described by Niewalda. Agarose plates were poured on the same day of the experiments. In contrast, Niewalda prepared the plates one day in advance and stored them overnight at room temperature. The reason behind this methodological change was based on concerns about possible diffusion effects.

According to the supervisor’s suggestion, extended storage time might allow NaCl to diffuse from the salt side into the pure agarose side, and therefore the intended concentration gradient could be changed. This could potentially change the sensory input for the larvae and affect their behavior and the results (Dr. Radostina Lyutova, personal communication). However, one point of agreement between the present study (**Figure 4**) and previous literature is the observation that very low NaCl concentrations tend to cause weak appetitive behavior rather than avoidance (Niewalda et al., 2008). The *ppk11* gene, which belongs to the *pickpocket* (ppk) gene family is essential for this mechanism and is expressed in specific gustatory receptor neurons (GRNs) responsible for detecting low NaCl concentrations (Liu et al., 2003). Another observation emerged from the first salt avoidance experiment under white light.

At the highest salt concentration tested (3.5M NaCl), the larvae showed no clear preference, neither for the salt side nor for the pure agarose side (**Figure 4**). However, this result was not supported by a subsequent, independently repeated salt avoidance experiment under identical white light conditions, in which a weak but still statistically significant avoidance of the salt side was observed (**Figure 5, 6F**). Therefore, the absence of preference at this concentration under white light is likely related to random variation or experimental noise rather than a meaningful behavioral effect.

Taken together, these findings highlight a key difference between these results and those reported in previous studies, the observation of an opposite linear relationship. In the next section possible reasons for this issue will be discussed in more detail.

4.3 Opposite Linear Relationship

Literature showed that a gene called *serrano* is important in high salt-sensitive GRNs for larvae to properly avoid high salt levels. In larvae lacking this gene, avoidance doesn’t work anymore, and they’re actually attracted to high salt concentrations (Alves et al., 2014). Furthermore, a spontaneous mutation could affect a properly functioning *serrano* gene, however, since spontaneous mutations are rare, it is highly unlikely that the observed behavioral pattern can be explained by a mutation. Although in the WT CantonS larvae that were used in this study, it could be possible, that the GRNs for high salt concentrations get saturated or overstimulated when exposed to the high amounts of salt, which might would explain why avoidance was so weak at higher concentrations in my experiments compared to Niewalda’s. Yet there is no literature describing this phenomenon.

In contrast to that, a different relationship occurred, when an experiment was conducted on testing larvae's naive gustatory behavior on different fructose concentrations. An "inverted U-shaped" response curve was observed (**Figure 7A, 7B**), which was actually expected and aligns well with the literature on this specific experiment (Schipanski et al., 2008). These findings and their implications will be discussed in more detail in the following chapter.

4.4 Sugar Preference under White Light

The sugar preference experiment revealed a clear inverted U-shaped response curve, where appetitive behavior increased with fructose concentration up to about 0.2–2 M, peaking at a PI of 0.6, before declining at higher concentrations, similar like in the salt avoidance experiments. However, like mentioned above, this result actually meets the expected result, gained by proven literature (Schipanski et al., 2008). At first glance, this might suggest that similar neurobiological mechanisms underlie the gustatory processing of salt and sugar in *Drosophila* larvae. One could hypothesize that comparable neurobiological pathways are responsible in processing high concentrations of both sugar and salt. However, established literature compete with this assumption (Schipanski et al., 2008) . While an inverted U-shaped-based behavioral response curve is indeed typical for sugar (fructose) (Schipanski et al., 2008), the behavior towards increasing NaCl concentrations is generally described with a steady linear rise (Niewalda et al., 2008). While in adult flies, certain gustatory receptor neurons expressing specific genes (*Gr5a* and *Gr64a*) are known to mediate sugar processing, it is still unclear whether these genes play a similar role in larvae, especially since reporter expression for these genes is often absent in larval stages (Colomb et al., 2007). Additionally, in larvae, sugar taste has been linked to the expression of *Gr43a* in the pharynx and the brain. (Maier et al., n.d.). Overall, despite the similarities of the results in the sugar and salt assay, the underlying gustatory pathways for salt and sugar likely differ.

4.5 Time-Dependent Effects on Naive Gustatory Behavior

In both the sugar preference and salt avoidance experiments, notable temporal effects on naive gustatory behavior were observed. Specifically, comparing shorter and longer observation periods revealed two consistent trends. First, the variability in behavior decreased over time. In the sugar preference experiment, the PI values showed less dispersion at 180 seconds compared to 90 seconds for the fructose concentration larvae showed the strongest appetitive response (0.2M, 2M). On top of that the overall appetitive response was higher at those fructose concentrations at the respective time points (**Figure 7A, 7B**). So, could it be that *Drosophila melanogaster* larvae require a certain amount of time to process gustatory input and make consistent decisions? This possibility is supported by established literature, where two 16 minute choice assays using 2M and 4M fructose concentration were conducted, in which larvae began to exhibit stable appetitive behavior after roughly 8 minutes, suggesting that gustatory decision-making in larvae may require a certain time to develop (Schipanski et al., 2008). Similarly, in the salt avoidance experiment however, the reduction in variability was less pronounced.

Larvae exhibited significantly stronger aversive behavior at 15 minutes in comparison to 3 minutes, at higher NaCl concentrations where avoidance was strong (0.5M, 2.5M) (**9C, 9D, 9E**).

Overall, these results suggest that larvae require a certain period of time to completely process gustatory information and make their final decision. A possible explanation for that could be, that they might begin in an “exploratory mode”, moving around and gather sensory input. Over time, the repeated exposure to the stimulus could lead to a clearer signal in their nervous system, and finally leading to a more confident choice. Another reason could be, that certain gustatory receptors get saturated after a certain time especially on sugar, giving them positive feedback and possibly reducing their movement, causing larvae to stay on the sugar side.

4.6 Summary

In conclusion, different lighting conditions had no effect on larval behavior, while longer exposure times tended to strengthen and stabilize the observed gustatory responses for certain concentrations. Last but not least, different concentrations of fructose and NaCl did indeed strongly affect the naive gustatory behavior of the larvae, even though not as expected, regarding salt avoidance. Although, the experiment was conducted according to the methods by Niewalda et. al., the results could not be replicated successfully. Therefore, the observed opposite pattern still cannot be explained. However, the successful replication of the sugar preference experiment by Schipanski et al. suggests, that there may be issues with salt processing in the CantonS strain used. To investigate this further, additional experiments are needed.

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5 Attachment

Table 1: Salt Avoidance Experiments: pairwise test results: *significance levels: p>0.05= n.s.; p<0.05= *; p<0.01= **; p< 0.001= ****

Figure	**Statistics**	**0M<0.025M**	**0.025M<0.5M**	**0.5M<1.5M**	**1.5M<2.5M**	**2.5M<3.5M**
4A	2S t-test	0.1081	<0.001	0.5952		
	Wilcoxon RS				0.5988	1
4B	2S t-test	1	<0.001	0.1160	0.2187	0.7685
5	2S t-test	1			0.7961	<0.001
	Wilcoxon RS		<0.001	0.0063		
6	2S t-test	1	<0.001			0.4032
	Wilcoxon RS			0.0046	1	

Table 2: Sugar Preference Experiment: pairwise test results: *significance levels: p>0.05= n.s.; p<0.05= *; p<0.01= **; p< 0.001= ****

Figure	**Statistics**	**0.002M<0.02M**	**0.02M<0.2M**	**0.2M<2M**	**2M<4M**
8A	2S t-test	0.0025	0.0099		
	Wilcoxon RS			1	0.0020
8B	2S t-test	0.0116	<0.001	1	
	Wilcoxon RS				0.0015

6 Acknowledgment

I would like to express my sincere gratitude to Prof. Dr. Björn Brembs - head of the department and examiner of my thesis - for his support and the opportunity to work on this project.

I am also deeply thankful to my supervisor, Dr. Radostina Lyutova for her expert guidance, valuable advice, and continuous support throughout my thesis.

I would also like to express my appreciation to Marcela, Daniel, Eva, Julia, and the entire lab team for their excellent teamwork and the help they gave me throughout the research.

Last but not least, I'm truly thankful to my family and friends for always being there, encouraging me, and supporting me throughout my studies.

7 Declaration of Authorship

The submitted printed copies and the submitted electronic version of the thesis are identical. I have written the thesis independently, have not used any sources or tools other than those indicated and have not already submitted the thesis to another university for the purpose of obtaining and academic degree. Furthermore, I confirm that I am aware of the legal consequences provided in § 27 (5) of the current examination regulations.

Die vorgelegten Druckexemplare und die vorgelegte elektronische Version der Arbeit sind identisch. Ich habe die Arbeit selbständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und die Arbeit nicht bereits an einer anderen Hochschule zur Erlangung eines akademischen Grades eingereicht. Weiterhin bestätige ich, dass ich von den in § 27 Abs. 5 der geltenden Prüfungsordnung vorgesehenen Rechtsfolgen Kenntnis habe.

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