Mathematical modeling of navigational decisions based on intensity versus directionality in *Drosophila* larval phototaxis

Lucia de Andres-Bragado, Christian Mazza, Walter Senn* and Simon G. Sprecher*

1Department of Biology, University of Fribourg, Fribourg, Switzerland
2Department of Mathematics, University of Fribourg, Fribourg, Switzerland
3Department of Physiology, University of Bern, Bern, Switzerland

*Corresponding authors

E-mail: simon.sprecher@unifr.ch (SGS), christian.mazza@unifr.ch (CM)
senn@pyl.unibe.ch (WS)

Short title: Light intensity and directionality drive *Drosophila* larval phototaxis

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Abstract

Organisms use environmental cues for directed navigation. Depending on the sensory modality and complexity of the involved sensory organs, different types of information may be processed. Understanding the basic logic behind navigational decisions critically depends on the complexity of the nervous system. Due to the comparably simple organization of the nervous system of the fruit fly larva, it stands as a powerful model to study decision-making processes that underlie directed navigation. Here, we formulate a stochastic method based on biased Markov chains to model the behavioral basis of negative phototaxis. We have quantitatively measured phototaxis in response to defined sensory inputs. We find that larvae make navigational decisions by taking into account both light intensities and its spatial gradients, and our model allows us to quantify how larvae minimize their exposure to light intensity and at the same time maximize their distance to the source of light. The response to the light field is a non-linear response and saturates above an intensity threshold. Our mathematical model simulates and predicts larval behavioral dynamics only using light intensity and directionality as input parameters. Moreover, it allows us to evaluate the relative importance of these two factors governing visual navigation. The model has been validated with experimental biological data yielding insight into the strategy that larvae use to achieve their goal with respect to the navigational cue of light, paving the way for future work to study the role of the different neuronal components in this mechanism.
Author Summary

Navigational decision-making is a complex process during which the nervous system is able to decipher external input through molecular and cellular mechanisms to produce a spatially-coordinated behavioral output. Drosophila larvae provide an excellent model to understand these decision-making mechanisms as we can measure the behavioral output (larval navigation) in response to quantifiable external input (different light conditions). We have performed experiments to quantify larval light avoidance in order to subsequently design a mathematical model that quantitatively reproduces larval behavior. Our results allow us to characterize the relative importance of light intensity and directionality and yield insight into the neural algorithms used in the decision-making mechanism of larval phototaxis.
Introduction

The nervous system is functionally organized to perceive external cues, which are encoded and decoded to make the correct behavioral decisions. A way of studying the logic of these decision-making mechanisms is through the analysis of robust stereotypical navigational strategies evoked by controlled stimuli (such as light or odor cues) in simple model organisms. Indeed, much of our understanding in the fundamental logic of taxis has been achieved by studying organisms such as *Escherichia coli, Caenorhabditis elegans* or larvae of the fruit fly *Drosophila melanogaster* (1, 2). Navigation in *Drosophila* larvae has been assessed in response to different types of single sensory inputs including vision (3, 4), olfaction (5, 6, 7, 8) and thermosensation (9, 10, 11) and a combination of inputs to study multisensory integration (12, 13).

*Drosophila* larvae show robust navigation towards appetitive and away from aversive cues. However, in the absence of an external cue or for a group of blind larvae, the dynamics is essentially random-like (14). In the presence of a light source, we experimentally show that the taxis is still moderately stochastic, being at most three times less efficient than ballistic dynamics. Therefore, an underlying Markov chain presents itself as an excellent basis for modeling larval dynamics since the external field (light) only introduces a small perturbation that allows a quasi-equilibrium description. That Markov chain is biased to take into account the external cue using Boltzmann’s probabilities (15). Measurable magnitudes can be obtained by averaging them over the simulated trajectories. Stochastic techniques like the one we are proposing here have been recently used to model taxis as a diffusion problem (16). The power of such stochastic
techniques rests on its capacity to tackle complex problems, like diffusion or phase transitions, with a moderate cost in computational time. In particular, the Metropolis-Hastings chain has been used to efficiently locate global minima of combinatorially-complex objective functions such as the *travelling salesman* problem (17). In this work, we show how to introduce generalized Metropolis-Hastings weights to bias a Markov chain to extract information from biological experiments where larvae take decisions using information gathered from their immediate surroundings.

Previous approaches to model taxis behavior have taken advantage of dividing the animal’s movements into a set of discrete behavioral states and to analyze the transitions between these states. One modeling approach has been based in a linear non-linear Poisson cascade to model the transition between the larval states (12, 13). Another approach has been to model larval taxis as continuous oscillations whose direction is not controlled by the stimuli but by an *intrinsic oscillator* and where the external stimuli would influence the amplitude of the oscillation (18).

*Drosophila* larval phototaxis provides an excellent model to study behavioral decision-making because their exposure to the sensory stimulus of light can be tightly controlled (3, 4). Their paths bear a strong connection with the intensity of light and to the position of the light source, which in the literature has been termed as light directionality (3). Larvae perceive light through a pair of bilateral eyes, which have been shown to be absolutely essential for visually-guided navigation (4, 19, 20, 21, 22, 23). Information from the external field of light is
then processed in the brain by a genetically hard-wired decision-making algorithm. Our model allows us to characterize that algorithm as a combination of a goal-directed behavior layer on top of a stochastic one.

By focusing on the impact of two key excitation elements, light intensity and light directionality, we present a model that studies the interplay between these two components for navigation. We exploit experimental navigational data obtained from various controlled illumination conditions to define probability weights that we use to polarize an underlying Markov chain that has been introduced to analyze larval taxis using stochastic methods. Such a mathematical model allows us to simulate larval dynamics with a minimal number of free parameters. Our model provides a theoretical and experimental framework of the decision-making mechanism functioning in the larval brain during navigation.

Results

Impact of light intensity and its spatial gradient on taxis

The navigation index (NI) has been used in the literature as a significant statistically-averaged dynamical parameter describing sensory guided navigation (3, 4). Along a given direction \( (x) \), the NI is defined as the mean velocity in that direction, \( v_x \), divided by the total velocity in any direction, \( v \). In other words, since velocities are measured over a common time interval, the NI in a certain direction can be taken as the distance moved in that direction, \( \Delta x \), divided by the length of the stratified path, \( s \). For example, the NI along the \( x \) axis, \( NI_x \), would be defined as \( NI_x = \frac{\Delta x}{s} \) (Fig 1A). Therefore, the value of the NI
provides an assessment of the efficiency of larval navigation. In our case, since
the source of light was located in the $+x$ axis of the agarose plate, the $NI_x$
would be approximately $-1$ for an object moving ballistically away from the
illumination source (negative values for the $NI$ imply that larvae navigate
towards the $-x$ axis). On the other hand, we would obtain a $NI_x$ of
approximately 0 for a random walk taken in the absence of an external cue or
for blind larvae.

Previous studies have shown that the intensity of light, its spatial gradient and
light directionality are relevant factors for visually-guided navigation (3).
Therefore, we hypothesize that these components may be sufficient to explain
the observed biological behavior in our defined experimental framework. First,
we experimentally tested the dependence of our primary dynamical measurable
magnitude ($NI$) with the two independent variables determined by the external
light field: the absolute light intensity and its gradient over the agarose plate
where the larvae are located (intensity is measured using irradiance units, as
the radiant power flux received per unit area, see Materials and Methods). For
this, we used a set of filters where light intensities and their spatial gradients
have been varied in a controlled and gradual way: f1, f2, f3, f4, f5 and f6 (Fig
1B). For all these experiments, the angle of the source of light was kept
constant at $40^\circ$. Our measurements show that navigation of wildtype larvae
depends on the absolute light intensity. Larvae show a very low navigation
score when the light intensity is low, such as in f1, where $NI_x = -0.03$. As the
light intensity increases, the value of the $NI$ increases in a non-linear way and it
saturates for intensities higher than $I > 20\,W/m^2$ to a value of around $NI_x = -0.3$
A heuristic expression that interpolates the dependence of the measured $NI$ with the absolute light intensity can be written as:

$$NI_x(I) = -0.28(1 - e^{-I/10})$$  \hspace{1cm} (1)

This interpolating function captures the two salient features of this experiment. Firstly, as expected for a dynamic process based on a Markov chain, it approaches zero in the absence of external stimulus ($I = 0$). Secondly, it saturates for around $I \approx 20 \text{ W/m}^2$ (Fig 1C).

Moreover, larval navigation also depends on the slope of the filter, $I'$ (Fig 1D). Same as for the light intensity, in the absence of a gradient, navigation is quite random-like, $NI (I' \approx 0) \approx 0$, and it shows saturation for gradients steeper than $I' > 0.20 \text{ W/m}^2$/cm (Fig 1D). A heuristic interpolating function that only depends on the gradient of the field of light is:

$$NI_x(I') = -0.28(1 - e^{-10I'})$$  \hspace{1cm} (2)

We do not assign the saturation of the $NI$ with the light intensity or its gradient to the physical response of larvae since the averaged speed at which larvae move takes a fairly constant value around $4 \text{ cm/min}$, being the small variation statistically non-significant (NS) and not obviously correlated to the intensity or the gradient of light (Fig 1E).

**Impact of directionality on phototaxis**

In a natural environment, light emitted from an external cue harbors directional as well as intensity information (3). To test the relative importance of these two components, we have projected a light pattern labelled as “Tilted” (Fig 2A) in which the intensity linearly decreases along the $y$-axis, thus perpendicular to
the light directionality along the \( x \)-axis. Contrary to the series of filters f1-f6, where both the light intensity and directionality drive larvae towards the same direction (\( x \)-axis), in the “Tilted” pattern, both effects are decoupled into two components: the light intensity artificially decreases in the \(-y\) direction because of the projected filter, while at the same time it naturally decreases along the \(-x\) direction as an effect of the increasing distance to the projector (S2 Fig 1).

Consequently, in the “Tilted” pattern, \( NI_y \) mainly accounts for larval navigation due to the variation of the light intensity, while \( NI_x \) could be taken as a proxy of the larval navigation away from the light source. We next quantified larval navigation along the \( x \)- and \( y \)-axis independently (Fig 2B). Larval phototaxis can be explained both by the light source avoidance (Fig 2B, “Tilted” \( NI_x \)) and by the avoidance of higher light intensities (Fig 2B, “Tilted” \( NI_y \)). However, in the “Tilted” pattern, light directionality (\( NI_x = -0.25 \)) has a stronger effect than intensity (\( NI_y = -0.07 \)) in driving negative phototaxis. On the other hand, even if in the case of wildtype larvae the light-intensity-driven \( NI_y \) value in the “Tilted” pattern is more than three times lower than the directionality-driven \( NI_x \) value, it is still statistically-significantly different from the \( NI_y \) of the visually-blind control \textit{glass}\textsuperscript{j60} homozygous mutant larvae, which completely lack eyes (\( NI_y\textit{glass}\textsuperscript{j60} = -0.01, p-value = 0.015 \)), proving that even if the wildtype \( NI_y \) for “Tilted” is small, it still remains light-intensity-driven taxis. Moreover, as expected, the \( NI_x \) for wildtype larval navigation in this “Tilted” pattern is also statistically-significantly different from the \( NI_x \) navigation of blind larvae (\( NI_x\textit{glass}\textsuperscript{j60} = -0.003, p < 0.001 \)). Therefore, we conclude that both the \( NI_x \) and the \( NI_y \)
navigation of wildtype larvae in the “Tilted” pattern are due to the visual system and not to other effects.

To further investigate the relationship between directionality and light intensity, we have generated a pattern labelled as “Pos” (for positive) where both effects reinforce each other in the same direction and another one where they compete in opposite directions along the \(-x\) and \(+x\) axis, labeled as “Neg” (for negative) (Fig 2A). We observed that navigation is stronger for reinforcing intensity and directionality cues (“Pos”, \(NI_x = -0.27\)) than for competing ones (“Neg”, \(NI_x = -0.18\)). This also supports our finding from the “Tilted” pattern that the effect of light intensity is weaker than the directional one, since the difference in navigation indexes between “Pos” and “Neg” is just -0.09 (Fig 2C, Table 1), which we interpret as the drive towards \(+x\) in “Neg” (intensity) only subtracting about one third of the drive towards \(-x\) in “Pos” (directionality and intensity).

Furthermore, the effectively blind glass\(^{60}\) mutant larvae have a \(NI\) that is statistically indistinguishable from zero (Fig 2B and 2C), which proves that besides the different patterns of light, all other conditions are kept the same in all these three experiments. Table 1 provides values for experimental and simulated navigation indexes for all the projected filters.

**Table 1.** Experimental and simulated navigation indexes for all the projected patterns of lights used in the experiments (f1-f6, “Pos”, “Neg” and “Tilted”).

<table>
<thead>
<tr>
<th>Filter</th>
<th>(NI_x)</th>
<th>(NI_y)</th>
<th>(ni_x)</th>
<th>(ni_y)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>f1</td>
<td>(-0.03 \pm 0.02)</td>
<td>(-0.03 \pm 0.01)</td>
<td>(-0.03 \pm 0.004)</td>
<td>(-0.001 \pm 0.005)</td>
<td>4.93</td>
</tr>
</tbody>
</table>
Experimental navigation indexes (dimensionless) in $x$ ($N_{I_x}$) and $y$ ($N_{I_y}$) directions, and the corresponding simulated navigational indexes, $n_{i_x}$ and $n_{i_y}$, for a given effective temperature $T$ (W/m²) using $f(\alpha) = 1 - \left(\frac{\alpha}{180}\right)^4$. The standard deviation of the experimental $NI$ was calculated for 10 experiments for each illumination condition with around 30 larvae each. The standard deviation for the simulated $ni$ was calculated with 30 simulations for each case.

**Simulation of taxis as a function of intensity and directionality**

Next, we propose a mathematical model for larval phototaxis that allows us to rationalize the experimental results presented above in a unified way. Such a model must take into account two significant facts: (i) in the absence of light the observed dynamics is well described by a Markov chain resulting in a random walk characterized by $NI = 0$, and (ii) even for the higher intensities, the $NI$ takes relatively low values, indicating that the external field only amounts to a small but non-negligible perturbation on the dynamics. Dealing with a perturbation has the distinctive advantage that we may assume quasi-equilibrium, same as in a diffusive regime on a physical system (24). Under
these conditions, we introduce weights in the underlying Markov chain to
describe the bias driven by the external field. To write an expression for these
weights, we use simple biological considerations operating on the larvae; in a
transition between states $r$ and $r'$ in the Markov chain, we define the following
weights:

$$W(r \rightarrow r') = \Delta I(r \rightarrow r') + \beta < I > f(\alpha(r \rightarrow r')),$$

with

$$f(\alpha) = 1 - \left(\frac{\alpha}{180}\right)^4$$

The first term of Eq (3) gives the difference in intensity experienced by larvae
while taking a step from position $r$ to $r'$, which depends on the gradient of the
intensity, $\Delta I = I(r') - I(r)$. The second term is meant to describe the directional
factor. This term is proportional to the average intensity $< I >$ (in units of
irradiance, W/m$^2$) for each projected pattern, as it deals with the increased
reaction of larvae against brighter sources of light, which is hinted by the
experimental results in Fig 1C. It carries an angular dependence through the
function $f$ of the direction $\alpha$ in the transition $r \rightarrow r'$ (Fig 3A). We have tried
different models for $f(\alpha)$ that will be discussed below (Materials and Methods,
Determination of $f(\alpha)$) and Eq (4) shows the one that yields a best fit to the
experimental angular distribution probabilities. Finally, $\beta$ is a free parameter that
allows us to balance the unknown relative importance between intensity and
directionality according to the experimental evidence. This parameter is
obtained from a specifically-designed pattern of light (“Tilted”, Fig 2A), where
the first term dominates the $NI$ in one direction, while the second term
dominates the $NI$ in a perpendicular direction. Such a pattern provides two
nearly independent experimental values for the $NI$ that can be used to fit the
The probability of accepting the new state \( r' \) is then defined by the Boltzmann factor, \( e^{-W(r \rightarrow r')/T} \) (Materials and Methods, generalized Metropolis-Hastings), where \( T \) is a parameter that, following a thermodynamics simile, plays the role of an effective temperature (measured in the same units as \( W, \text{W/m}^2 \)). We find that the larval effective temperature has higher values for more intense light fields (f6 compared to f1, Fig 3B). We interpret the consequences of this behavior in the Discussion section. The error bar for \( T \) corresponding to the darkest filter (f1) is larger than for the other cases because in the absence of light, the larval movement ceases to be targeted and becomes similar to a random walk, where \( T \) is not meaningful anymore and cannot be determined. In mathematical terms, the effective temperature is obtained from a probability that depends on the quotient \( \frac{W(r \rightarrow r')}{T} \). In the absence of an external field we have \( W = 0 \), and any value of \( T \) corresponds to the same probability so its value is not well defined (unbiased Markov chain).

The biased Markov chain is not time reversible (principle of detailed balance) since it is driven by an external field that imposes a definitive direction over time. However, in a similar process to the equilibration pattern followed by a thermodynamics system, we have found both in our simulations and in our experiments that important dynamical indicators, like the \( N/V \), reach a steady state after some initial fluctuating steps. Therefore, these indicators converge to a well-defined value and can be safely compared between simulations and experiments.
The angular part in $W$, $f(\alpha)$, is a dimensionless function chosen to obtain the best possible fit to the experimental angular probability distributions. We have found that a good choice for $f(\alpha)$ is to make it proportional to a power of the angle $\alpha^n$, where $\alpha$ is the angle formed in the plane of the agarose plate between the attempted direction $\mathbf{r}'$ and the $x$ axis (Fig 3A), and the power $n$ can be taken as a free parameter that is chosen to obtain the best fit to experiments. In particular, we have found that $n = 4$ is an optimal value. Possible choices for $f(\alpha)$ are described in more detail below (Materials and Methods, Determination of $f(\alpha)$).

Our mathematical model yields more targeted paths when the intensity and its gradient are higher (Fig 3C, f6 compared to f1) which leads to higher values for $NI$. Furthermore, simulated larval paths look similar to the experimental ones (Fig 3D), which indicates that not only averaged values like the $NI$ can be successfully compared between simulations and experiments. In fact, the similarity of the angular distribution for the experimental larvae and the simulated ones proves that our model quantitatively reproduces experimental results under different light conditions; Fig 3E shows the agreement between experimental (grey curves) and simulated (green curves) angular distributions obtained with the non-linear $f(\alpha) = 1 - \left(\frac{\alpha}{100}\right)^4$ for filters f1 to f6.

**Discussion**

**Larvae respond differently to different intensities and light gradients**
Our results show that larval navigation depends on light intensity and its gradient and that larvae navigate more efficiently (larger $NI$) when the light intensity is higher and when the gradient of the intensity is steeper. However, this non-linear behavior (Eqs (1) and (2)) saturates for intensities higher than $I > 20 \text{ W/m}^2$ (Fig 1C) and for intensity gradients higher than $I' > 0.2 \text{ W/m}^2/\text{cm}$ (Fig 1D). As commented above, we do not assign such a saturation behavior to a lack of physical response from the larvae since the mean velocity is nearly independent of the illumination conditions, but rather to a limited capacity for processing information in the underlying neural network in the larval brain. From a biological point of view, larvae make decisions to take a step from position $\mathbf{r}$ to $\mathbf{r}'$ or not, based on the local conditions surrounding them. These conditions are the only ones that larvae can probe with their limited visual organ and the only ones that they can process in their brains without requiring an expensive memory process to record a string of magnitudes all along their paths. Saturation regarding the light intensity can be understood from a limited ability to process the input signal of too many photons. On the one hand, the experimental evidence that larvae navigate differently depending on the gradient of the intensity implies that larvae must read the gradient of the field of light ($I'$). Such an operation needs to measure the intensity in two close points and then proceed to compare them. Therefore, it involves a memory process if it is to be done at two subsequent times along the larval path.

Similarly, in the model, whether a transition in the Markov chain happens or not is based on variables that can be locally obtained using only the larval starting position.
Distinct larval directional tendency to move away from the light source

The combination of our experiments with our mathematical model shows the detailed relationship between light intensity and directionality for larval phototaxis for the first time. The analysis of the “Tilted” pattern by our mathematical model tells us that the main reason for larval phototaxis, in about a one to three ratio, is to get away from the source of light, rather than to simply move to darker regions. This is in part due to the saturation process shown in Fig 1C and 1D, that limits the effect of the first part in Eq (3). The weights defined in our model show, in a quantitative way, that larvae can process the relative orientation of light. Such a capacity to discriminate different angles explains the larval ability to move maximizing the distance to the source of light by simply giving a higher probability to angles around $180^\circ$ (away from the light source) than to angles around $0^\circ$ (towards the light source).

Larval phototaxis and the proposed mathematical model

An interesting feature of our mathematical model is that it only requires three adjustable parameters: the relative balance between intensity and directionality $\beta$, Eq (3); the power $n$ telling how sensitive the larvae is to changes in the light direction, Eq (4); and the effective temperature $T$ that determines the stochastic exploration (see Eq 5 below). The reason for needing so few parameters is probably linked to the general principles governing the generalized Metropolis-Hastings algorithm, which takes care of the statistical behavior in a way that is known to work well for many different complex systems found in nature. The
model is based in principles so well accepted in different contexts that except for particular details in Eq (3), it should work for other organisms and other sensory cues.

The related Metropolis-Hastings algorithm has been successfully used to efficiently locate *global* minima of combinatorially-complex objective functions such as the travelling salesman problem (17). In contrast, we remark that our biological experiments mostly bring information about larval decisions taking into account *local* data (intensities and gradients in the immediate surroundings of the organism) and proceed with a limited amount of neural circuitry. Therefore, the weights governing the simulation in Eq (3) should be considered as a local solution to the problem rather than a global one.

The value of the effective $T$ in the simulations is adjusted so that the currents of larvae going towards the light source and in the opposite direction match the experimental $NI$ measured under some particular light conditions. Therefore, this $T$ determines a quasi-equilibrium condition, similar to the one found in a chemical reaction where reactants convert into products, and vice versa, in ratios that match the actual production at a given temperature. On the other hand, such a parameter lends itself to a biological interpretation; it controls the larval probability of taking risks by either going to higher intensity regions or by getting closer to the source of light. Such a behavior is known to be a useful way to avoid being trapped in local minima, as it has been proved when simulated annealing has been applied to find the global minimum of a given objective function. Our results show that the effective temperature grows with light intensity and its gradient. These are the conditions that from a biological
point of view should require more vigorous action from the organism to quickly find a more convenient position. In turn, when conditions are not so harsh, organisms prefer taking conservative decisions, hardly moving to worse regions in order to explore their environment more efficiently.

Regarding the different models that we have tried for $f(\alpha)$, the functions that reproduce the experimental data better are the power-like ones. Linear functions of the angle do not agree well with experiments; all reasonable candidates have been highly non-linear functions. Therefore, the larval behavior reveals a complex and rich neural network behind the process of taking decisions, which works on a non-linear function, which is a common feature to neural circuits organized in layers (25).

So far, the model does not take into account the larval dimensions. However, it would be possible to add terms to the weights $W(r \rightarrow r')$ in Eq (3) to take into account the size of the larva, for example, by artificially increasing the value of these weights when two larvae would overlap on the new position $r'$. In this case, we could take into account the fact that they cannot go to places already occupied by other larvae and even the attraction or repulsion between individuals could be modeled (26, 27). This would open the possibility to study simulated group behavior, although at a higher computational cost.

An additional feature of larval taxis, studied for chemotaxis, is weathervaning, which is defined as miniature head-sweeping during runs resulting in curved tracks (28). Whether weathervaning plays a role in phototaxis or not remains
unclear. Since our mathematical model is based on the local values of weights for the underlying Markov chain, *weathervaning* is not directly taken into account. The model is based on larval runs and turns with a single underlying mechanism governed by the weights in Eq (3). Moreover, (29) have studied *weathervaning* for larval chemotaxis to conclude that it is the least crucial navigational parameter according to their model.

Relevance for the neuronal network involved in larval phototaxis

Mathematical models of larval navigation provide the first step towards understanding the underlying mechanisms that operate in the larval neuronal network to lead to decision-making. Next steps in understanding the neuronal basis of visual navigation may include to combine current information of the connectome with behavioral data and to correspondingly adapt a mathematical model (30, 31). This generalized Metropolis-Hastings-based model could also be used for other stimuli, the only requirement being that the intensities and gradients of these stimuli should be measured and quantified properly to define the details of the weights in Eq (3). Once these weights have been found for other stimuli, multi-sensory experiments could be carried out to see if these factors are additive towards larval navigation as suggested in (12).
Materials and Methods

Fly strains

Wild-type Canton S (WTCS) *D. melanogaster* larvae (courtesy of R. Stocker), and *glass*mutants (Bloomington 509) were used for these experiments. All the fly stocks were kept at 25°C in a 12-hour light-dark cycle. The stocks were fed with a conventional cornmeal medium containing molasses, fructose and yeast.

Behavioral experiments

Larvae were selected for experiments after four days since the egg-laying of the parental flies, ensuring that they would correspond to the 3rd larval stage (L3). Larvae were kept for at least 10 minutes in the dark with food before the phototaxis experiments were carried out. Thirty larvae were isolated from the food for each experiment and placed in water droplets with a paintbrush. The maximum time for the larval selection was 10 minutes and it was done under red light conditions. The larvae were left in the agarose plate without food and their tracks were recorded for 11 minutes. The first minute was not taken into account to let the larvae get used to the new conditions. The behavior experiments were always carried out within the larval 12 light-hours.

Tracking system

The experimental setup consists of a 23x23 cm agarose plate where the larvae can move freely (Fig 1A). Larval movements were recorded with a Basler acA2500-14gm camera equipped with a 1:14/12.5 mm Fujinon lens and placed directly above the tracking arena. The lens was incorporated with a red filter.
(635 nm, Qualimatest SA, Geneva, Switzerland). The agarose plate was illuminated with red LEDs that do not influence larval behavior but enable the image recollection with the camera (Fig 1A).

An EB U04 projector was located at \( x = 36.5 \text{ cm}, \ y = 0 \text{ cm}, \ z = 25 \text{ cm} \). The projector was equipped with a 2" Square BG40 coloured glass bandpass filter 335 – 610 nm and was placed forming a 40° angle with respect to the \( x - y \) plane formed by the agarose plate (zenithal angle, \( \theta \), Fig 1A).

The custom-made LabView software (32, 3) was used to record the larval movies.

**Light intensity measurement on the agarose plate**

Different light patterns were projected to obtain the different experimental scenarios against which the simulations could be validated. The intensity field varied in a different way in all of them. f1-f6 were uniform filters where the intensity variation was merely due to variation of the photon flux with the distance to the projector (Fig 1B and S1 Fig 1). In the patterns “Pos”, “Neg”, and “Tilted” used to explore directionality, an artificial modulation in the light gradient along the \( x \) or \( y \) directions was introduced and therefore there was a steeper change in light intensity. In “Pos”, the maximum light intensity was closer to the light source, same as in all the f1-f6 patterns, but the light intensity decreased along the \(-x\) axis with a gradient that was about 5 times steeper (S1 Table 1 and S2 Table 1). “Neg” was a 180° rotation of “Pos”; therefore, the intensity field decreased along the \(+x\) axis and the brightest area was located further away.
from the light source. “Tilted” was a 90° rotated version of the “Pos” pattern. In this case, the light gradient artificially varied along the y direction, therefore the intensity field decreased along the \(-y\) axis (Fig 2A, S2 Fig 1C).

Light intensities created by the different projected patterns were measured on the agarose plate using an Ocean Optics USB400 spectrometer. Three equally-spaced points along the \(x\) axis (\(y = 0\)) were measured for the filters used to study light intensity (f1-f6, S1 Fig 1) and nine points equally covering the \(x\) and \(y\) directions for the patterns related with directionality (“Pos”, “Neg”, and “Tilted”, S2 Fig 1). Several measurements were taken for each point on different days and standard errors were calculated. The total intensity (W/m²) was obtained by integrating these spectra between 380 and 570 nm to include the blue and green wavelength regions of the spectrum relevant for Rh5 and Rh6 absorption spectra, but to exclude the red one (S1 Fig 1 and S2 Fig 1). Integrals have been performed by first defining an interpolating polynomial going through all the experimental points, and then using an accurate Gaussian-Konrod rule for integration (33).

The expected variation of light intensity on the plate by a uniform source of light is described by assuming a steady rate of generation of photons. For the actual parameters of the geometrical setup, this has the implication of an approximate linear variation, which has been corroborated by measuring intensities on the plate (S1 Fig 1 and S2 Fig 1). Therefore, the spatial variation of intensities has been represented by a linear fit with

\[
I(x, y) = a_0 + a_{1x} + a_{1y} \tag{5}
\]
where $a_0$ is the value at $x = y = 0$ and $a_{1x}$, $a_{1y}$ are the slopes along the $x$ and $y$
directions. Values for these coefficients are given in S1 Table 1 and S2 Table 1.

The air conditioning was turned on at 25°C during the experiments to ensure a
constant temperature in the agarose plate. Measurements of the temperature
on the plate always yielded temperatures in the interval between 25°C and
26°C.

**Tracking data analysis**

The acquired images of the larval tracks were analyzed with the MAGAT
Analyzer (3). The features of each larva (head, tail and midline) were extracted
from the videos and these data were analyzed using a custom-made software
written in MATLAB (34).

Statistical analysis of the data was calculated using the Welch's unpaired $t$-test
to compare results with different genotypes and a regular unpaired $t$-test was
used to compare larvae with the same genotype. The Benjamini-Hochberg
procedure was applied to correct for multiple comparison. The statistical
difference of results compared with zero was calculated using a one-sample $t$-

**Generalized Metropolis-Hastings chains**

In our simulations, we assign transition probabilities between states in the
Markov chain according to the Boltzmann distribution. Probabilities are
assigned in the following way (35):
1. A description of possible system configurations and the options presented to the system. These are determined by giving:

   a. The initial position of the larvae, \( r = (x, y) \) and the final attempted position, \( r' = (x', y') \), where \( x' = x + \Delta x \) and \( y' = y + \Delta y \)

   b. The light intensity at both points: \( I(r) \) and \( I(r') \)

2. A generator of random changes in the configurations. We chose the next position using two independent Gaussian deviates with zero mean (\( \Delta = 0 \)) and standard deviation one (\( \sigma = 1 \)) for the independent increments in the \( x \) and the \( y \) direction, \( \Delta x \) and \( \Delta y \) respectively. This defines a discrete-time continuous-space Markov chain of transition kernel \( k_0(r; r') = e^{-\frac{1}{2}r'^2-r^2} \).

   The standard deviation sets up a length scale that we adjust to the observation that the larvae approximately advance a distance equivalent to the length of its body in about ten moves. Therefore, the standard deviation is equivalent to approximately 0.1 mm.

3. The larval local moves are described by a discrete-time continuous-space Markov chain of transition Kernel:

   \[
   k(r \rightarrow r') = k_0(r \rightarrow r') \times e^{-\frac{W(r \rightarrow r')}{r}}
   \]  

   where \( W(r \rightarrow r') = \Delta I(r \rightarrow r') + w \) and \( I > f(\alpha(r \rightarrow r')) \). Algorithmically, the new \( r' \) is chosen as follows:

   - Choose \( r' \) according to the Gaussian model \( k_0 \), \( r' = r + \mu \), where \( \mu \) is a bivariate Gaussian deviate with zero mean (\( \Delta = 0 \)) and standard deviation (\( \sigma = 1 \)) in both dimensions
   
   - If \( W(r \rightarrow r') < 0 \), then \( r' \) is accepted with probability \( P = 1 \)
• If $W(r \rightarrow r') > 0$, then $r'$ is accepted with probability $P = e^{\frac{W(r \rightarrow r')}{T}}$.

4. A control parameter $T$. This parameter controls the weights so that the simulation can reproduce the experimental navigation index for a given light intensity pattern. $T$ has irradiance units, same as $W$, and in a thermodynamics system it would be the equilibrium temperature. High values of $T$ leads to small values of the exponential weight $e^{\frac{W(r \rightarrow r')}{T}}$, and the related transition step will occur with a probability near to 1 for positive $W$.

The experimental recording stops tracking larvae that hit the border of the agarose plate. Accordingly, we introduce a similar boundary condition in our simulations and we stop tracking larvae that after $N$ accepted steps have reached a distance to the origin greater or equal to $1150 \sigma \approx 11.5$ cm. Depending on the illumination conditions, this usually happens after a few thousands accepted steps. At that point, the Markov chain has an absorbing state. However, we checked that the averaged $NI$ and the angular probability distributions reached a quasi-stationary state before the experiment is terminated. For a given light pattern, each simulation was carried out with 30 individual larvae, and the average $NI$ and its standard error have also been computed for a 30 larvae ensemble, making sure that the given $NI$ is statistically significant.

**Determination of $f(\alpha)$**

The angular part in equation (3) has been modelled to take into account the experimental angular distributions. The angle $\alpha$ is measured with respect to the
\[ x \text{ axis, being } 0^\circ \text{ the direction towards the projector and } 180^\circ \text{ the direction away from it (Fig 3A). Two types of models for } f(\alpha) \text{ were tried: power-like models proportional to } \alpha^n \text{ and models based on } \cos^n(\alpha), \text{ taking into account that } \\
\cos(\alpha) = \Delta x / \Delta l \text{ (S3 Fig 1). Each model was assessed calculating the standard deviation of the angular probability distribution of the simulated paths compared to the experimental ones. Both the experimental and simulated paths were binned in } 30^\circ \text{ angles and the probabilities for both experimental and simulated cases were compared. The best fit to experiments across all the different projected patterns was found for } f(\alpha) = 1 - (\alpha/180)^4, \text{ as shown in S3 Table 1, where we give the root-mean-squared (RMS) deviation between experimental and simulated angular distributions for all models tried for } f(\alpha). \]

**Determination of \( \beta \)**

The value for the parameter \( \beta \) (\( \beta = 1.4/100 \)) was determined taking the “Tilted” pattern as a case where the two terms of the objective function (intensity and directionality) are most decoupled. Consequently, the \( NI_y \) in the “Tilted” pattern was simulated assuming that only the first term in the objective function would exist. That procedure yields a value for the effective \( T \). Afterwards, the value for \( NI_x \) was used to find a value for the parameter \( \beta \). As a final consistency check, both the \( NI_y \) and the \( NI_x \) were simultaneously recalculated using the two parts of the objective function obtaining a refined value for \( T \) that fits the two available experimental values at the same time.
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Figure captions

Fig 1. Larval navigation depends on the absolute light intensity and the gradient of the light field. (A) Experimental set up formed by the agarose plate ($x-y$ plane), light source (projector located at $x = 36.5$, $y = 0$, and $z = 25$), forming an angle $\theta = 40^\circ$ with respect to the agarose plate, and a video-recording camera. The larvae move on the agarose plate and their positions are recorded by the camera. The LEDs placed on the border of the plate aid in the image-acquisition process. The navigation index in the $x$ direction ($NI_x$) is calculated by dividing the distance moved in the $x$ axis, $\Delta x$, by the total length of the stratified path, $s$. (B) Filters with different light intensities and gradients, f1 – f6 (S1 Fig1 and Table 1). (C) $NI_x$ and standard deviation plotted against the average intensity over the plate for each filter, <$I>$ (W/m²); the resulting curve shows that the efficiency of larval navigation depends on <$I>$. The dashed line is a least-squares interpolation to data used to guide the eye (see text). The values for the different filters were compared using the Welch t-test and Benjamini-Hochberg was used to correct for multiple comparison. (D) Same as (C) as a function of the gradient for each filter, $I'$ (W/m²/cm²). (E) Larval mean run speed for the filters f1-f6 takes values from 4.0 cm/min to 4.5 cm/min, which is a statistically non-significant difference (NS). The whiskers of the boxplots represent the range of the mean run speed for the different filters. Within the boxplots for each filter, the middle band is the median (50th percentile), and the length of the boxplots shows the 1st and 3rd quartile (25th and 75th percentile).
Fig 2. Light directionality plays a big role in larval navigation. (A) Projected filters “Pos”, “Neg” and “Tilted”, used to study the joint effect of light intensity and light directionality. All these filters were projected individually in the agarose plate and the light source was always in the +x side of the agarose plate as shown in Fig 1. In “Pos”, the light intensity is higher closer to the projector (right, +x) and decreases along the −x axis (increasing distance to the projector). “Neg” is the same filter but rotated 180°, where the light intensity increases as the distance from the light source increases. “Tilted” is a 90° rotation of the “Pos” filter: light intensity increases in the +y direction. (B) Wild-type Canton S (WTCS) Larvae in the “Tilted” filter (dark purple boxplot) have a statistically-significantly different navigation index both in the x direction ($NI_x = -0.25$, $p < 0.001$) and in the y direction ($NI_y = -0.07$, $p = 0.0152$) compared with the effectively blind glass mutants navigating in the same filter (grey boxplot). The directionality effect (measured in “Tilted” by $NI_x$) is stronger than the light intensity effect (measured by $NI_y$) as most of the larval navigation is in the x axis ($p < 0.001$). The $NI$s were compared using the Welch t-test and corrected for multiple comparison with the Benjamini-Hochberg procedure. The range of $NI$s is given by the whiskers of the boxplots. The median (50th percentile) is represented with the middle line and the 25th and 75th percentile are represented by the lower and top bands of the boxplot respectively. (C) Navigation index in the x axis ($NI_x$) (left graph) and in the y axis ($NI_y$) (right graph) for the filters showed in (A) (“Pos” in blue, “Neg” in green and “Tilted” in pink) for both WTCS (dark purple boxplots) and the blind glass mutant larvae (grey boxplots). Larvae presented with the “Pos” filter have the highest navigation index ($NI_x = -0.27$), as both the light intensity and light directionality
drive larvae to navigate in the $+x$ direction. Larvae navigating in the “Neg” filter have a lower navigation index ($NI_x = -0.18$) as both effects are driving them to navigate in opposing directions (light intensity towards $+NI_x$ and light directionality towards $-NI_x$). p-values are calculated with the Welch’s t-test and using the Benjamini-Hochberg procedure to correct for multiple comparison, where $p > 0.001$ is represented by ***, $p > 0.01$ ** and $p > 0.05$ by *. 
Fig 3. The model yields simulated larvae with similar navigation characteristics to the experimental larvae. (A) Coordinate axis used within the agarose plate to define larval movement. The angle $\alpha$ was defined within the $x - y$ plane of the agarose plate with respect to the $x$ axis, where 0° and 180° are the direction towards and away from the light source respectively. For the analysis, both the experimental and the simulated larval angular probability distributions, $P(\alpha)$, were binned in 30°. (B) The effective temperature $T$ (W/m²) increases proportionally to the average intensity $<I>$ (W/m²) of the light field. The dashed line is a linear interpolation to guide the eye. The error bars for the effective $T$ have been calculated using the experimental error for the navigation index ($NI$) and calculating the $T$ for $NI$ plus and minus this error. (C) Simulated larval paths for f1 and f6. Each path shows one simulated larva, which starts at (0,0) and moves towards the $-x$ side of the plate, avoiding the projector which is located on the $+x$ side of the agarose plate (right hand side). The model yields simulated larval paths reflecting the stochastic underlying Markov chain, but also targeted navigation to get away from the light source and from the regions of high light intensities. Taxis is more targeted for higher intensity conditions (f6) same as observed in the experiments. (D) Experimental larval paths for the f6 filter (30 experimental larvae are shown); these paths are similar to the simulated ones seen in (C). (E) Comparison of the relative probability of orientation with respect to the light source (located at the right, at 0°) both for simulated (green) and experimental (grey) larvae for the different light conditions (f1, f2, f3, f4, f5 and f6). Larvae are oriented at 180° when they navigate away from the light source, and at 0° when they navigate towards the light source. The probability of orientation for both the experimental and
simulated larvae has been calculated binning the possible angles in 30° bins.

The simulated larvae have been calculated using \( f(\alpha) = 1 - \left( \frac{\alpha}{180} \right)^4 \). Error bars are shown for the experimental angular distributions and they were obtained from the Matlab output files from the MAGAT Analyzer.
**Supporting information**

**S1 Fig 1. Light spectrum along the agarose plate for the f1 – f6 filters.** (A-F)

The light intensity was measured with an OceanOptics USB400 spectrophotometer in the agarose plate in three points for the f1 – f6 filters. Light intensities were measured for all the filters along the x axis of the agarose plate at three points: (1) one closer to the projector, at (11.5,0) cm (white square), (2) in the center of the agarose plate (0,0) cm (gray square) and (3) in the point furthest away from the projector (−11.5,0) cm (black square). Replicates of each of the measurements were taken on different days. (A’-F’)

Light intensity (I) is given in μW/cm²/nm as a function of the wavelength λ in nm. The light intensity measured on these three points was plotted and integrated between 380 nm and 570 nm (vertical lines), which is the biologically relevant wavelength range for the larvae. (A’’-F’’)

The variation of light intensity along the x axis of the agarose plate was modelled with a linear regression for each of the filters.

**S1 Table 1. Light measurements for the f1 – f6 projected filters.** The three measured points along the x axis of the agarose plate for filters f1 – f6 (S1 Fig1 A-F) were used to model the variation with a linear regression. Light intensity is quantified with a least-square polynomial fit to intensities. The slope of the linear variation of intensities (a₁x in W/m²/cm) and the intercept (a₀ in W/m²) can be seen in this table for each of the filters.

**S2 Fig 1. Light spectrum along the agarose plate for the directionality patterns.** (A-C) The light intensity was measured as in S1 Fig 1 for the “Pos”,
“Neg” and “Tilted” patterns in nine points forming a homogenous grid in $x$ and $y$.

The gradients of light intensities along the $x$ axis and along the $y$ axis were obtained from that grid. Replicates of each measurement were taken on different days. (A'-C') Same as in S1 Fig 1 for “Pos”, “Neg”, and “Tilted”. (A''-C'') Variation of light intensity along the $x$ axis on the agarose plate for “Pos” and “Neg” and along the $y$ axis for “Tilted”. A linear regression for each of the patterns was found to describe well the pattern of intensities. (A'''-C'''') Contour plot for each of the projected filters.

S2 Table 1. Light measurements for the projected directionality patterns.

Light intensity is quantified with a least-square polynomial fit to intensities, $a_0 + a_{1x}x + a_{1y}y$. This table shows the values for the linear regression parameters: the slope of the linear variation of the light intensity along the $x$ axis ($a_{1x}$ in W/m$^2$/cm), along the $y$ axis ($a_{1y}$ in W/m$^2$/cm) and the intercept ($a_0$ in W/m$^2$) for each of the projected patterns.

S3 Table 1. Comparison of the different models for $f(\alpha)$. Simulations were carried out using different models for $f(\alpha)$ and tested against the f1 – f6 filters. Simulations for each model were carried out 30 times and the experimental ones were calculated doing 10 experiments with around 30 larvae each. Both the experimental and simulated angular probability distributions were binned in 30° angles. Each model was assessed by calculating the root mean squared deviation (RMS) between the experimental and simulated angular probability distributions for the different binned angles from 0° to 180°. Then, the average
of the RMS for all the filters was compared for each model of $f(\alpha)$. The smallest overall RMS was obtained with $f(\alpha) \propto 1 - \alpha^4$.

**S3 Fig 1. Geometrical diagram for the directionality part of the cost function.** The source of light is approximated by a plane, F. The light came from right (+$x$) to left (−$x$). The angular distribution is a function of the angle of the direction of the larva, $\alpha$, which is a function of the displacement $\alpha = \arctan \frac{\Delta y}{\Delta x}$. The increment in the distance to the plane F depends on the displacement $\Delta x = \Delta l \cos \alpha$, where $\Delta l = \sqrt{\Delta x^2 + \Delta y^2}$. 
Figure 1

A. Camera and Projector with Red LEDs

B. Color Gradient

C. Graph showing navigation index vs. light intensity

D. Graph showing navigation index vs. light intensity per unit area

E. Graph showing mean run speed for different conditions

Note: The image includes a legend indicating the significance levels for the data points (p-values).
Figure 2

A. Diagram showing the orientation and intensity of WTCS glass for different positions: Pos, Neg, and Tilted. The projector is tilted at an angle of $\theta = 40^\circ$.

B. Box plots showing Navigation Index (NI) for "Tilted" conditions in the x and y directions. "Tilted" NI $x$, "Tilted" NI $y$, "Pos" NI $x$, "Neg" NI $x$, "Tilted" NI $x$, "Pos" NI $y$, "Neg" NI $y$, "Tilted" NI $y$.

C. Box plots showing Navigation Index for different conditions: "Pos" NI $x$, "Neg" NI $x$, "Tilted" NI $x$, "Pos" NI $y$, "Neg" NI $y$, "Tilted" NI $y$. Statistical significance indicated by * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

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Figure 3

A. Camera and Projector

B. Graph showing T (W/m²) vs. <I> (W/m²)

C. Diagram showing f1, f6, and θ° = 40°

D. Diagram showing θ° = 40° and 0°

E. Graphs showing T (W/m²) for f1, f2, f3, f4, f5, and f6