Habituation in mosquito larvae *Aedes aegypti* is context-specific

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Summary statement: Larvae of *Aedes aegypti* were habituated to a visual stimulus in different visual contexts. Once habituated, larvae reacted to the same stimulus presented in a different visual context, proving that habituation was context-specific, i.e. there was an association between the context and the visual stimulus.

Abstract

Mosquito larvae live in water and perform a stereotyped escape response when a moving object projects its shadow on the surface, indicating potential risk of predation. Repeated presentations of the shadow induce a decrease in the response due to habituation, a form of non-associative learning defined as the progressive and reversible decrease in response to a specific reiterative innocuous stimulus. Nevertheless, habituation can be context-specific, which indicates an association between the context and the stimulus. The aim of this work was to study context-specificity in habituation in mosquito larvae *Aedes aegypti*. Larvae were individually placed in Petri dishes. Underneath, black, white or black-white striped cardboards were placed as backgrounds (visual *context*). Larvae were presented with a shadow produced by a cardboard square (*training*) over the course of 15 trials. After the fifteenth trial, the background was shifted and the stimulus was presented once again (*test*). To analyse habituation in different contexts, we developed a series of learning curve models. We performed a Bayesian model selection procedure using those models and the data from the experiments to find which model best described the results. The selected model was a Power-Law learning curve with six parameters (*habituation rate, context-specific asymptotic habituation response -with*

one parameter per context, i.e. 3 parameters-, *response-increase*, and *autocorrelation*) describing the whole experimental setup with a generalised r^2 of 0.96. According to the model, a single habituation rate would indicate that habituation was independent of the context, whilst asymptotic habituation would be context-specific. If the background was shifted after *training*, there was an increase in the response in the test, evincing context specificity in habituation.

Introduction

Learning is crucial for any animal, since it allows adapting the individual's behaviour to changing environments (Giles & Rankin, 2009). Furthermore, being able to recognise and to differentiate among different kinds of stimuli, reduces energy cost and time consumption (Scott, 2005). Habituation is a non-associative type of learning that allows filtering out irrelevant stimuli. When an individual faces a repetitive stimulus without any consequence, that stimulus becomes irrelevant. Consequently, a gradual decrease in its behavioural response occurs (Thompson & Spencer, 1966; Groves & Thompson, 1970; Giles & Rankin, 2009; Rankin *et al.*, 2009; Rankin, 2009; Klein, 2012).

Habituation has often been considered among the simplest and most elementary forms of learning. It has been documented in a wide diversity of animals, both vertebrates and invertebrates. Among vertebrates, habituation has been studied in horses (*e.g.* Christensen *et al.*, 2010), rats (*e.g.* Askew, 1970), birds (*e.g.* Dong & Clayton, 2009), fishes (*e.g.* Best *et al.*, 2008), rabbits (*e.g.* Whitlow, 1975), cats (*e.g.* Groves & Thompson, 1970), dogs (*e.g.* Pullen *et al.*, 2012), humans (*e.g.* Dycus & Powers, 1997). Among invertebrates, habituation has been studied in the nematode *Caenorhabditis elegans* (*e.g.* Rankin, 2000), molluscs (*e.g.* Pinsker et al., 1970; Carew & Kandel, 1973; Bailey *et al.*, 1983; Fisher *et al.*, 2011), crabs (*e.g.* Bruner & Maldonado, 1988; Lozada *et al.*, 1990; Tomsic *et al.*, 1998, 2009; Hemmi & Tomsic, 2012; Raderschall *et al.*, 2011) and insects (Tachind fly *Drino bohemica,* Monteith, 1963; honey bee *Apis mellifera*, Braun & Bicker, 1992; Gerber & Menzel, 2000; fruit fly *Drosophila melanogaster,* Glanzman, 2011; Soibam *et al.*, 2013; Twick *et al.*, 2014). More recently, habituation-like behaviour has been reported in the myxomycete slime mould *Physarum polycephalum* (Boisseau *et al.*, 2016).

The adult mosquito *Aedes aegypti* is the vector of several human diseases as dengue, yellow fever, Chikungunya, Zika and Mayaro viruses (Marklewitz & Junglen, 2019). Pre-imaginal stages of this holometabolous insect, *i.e.* from egg to pupa, develop in clean and calm bodies of water. When confronted to a moving visual stimulus above the water surface (*e.g.* a shadow), mosquito larvae and pupae perform a stereotyped escape response (fast movements with the abdomen letting the animal dive; Holmes, 1911; Thomas, 1949; Leftwich, 1954; Mellamby, 1958). The escape response causes significant energy expenditure affecting survival and fertility (Timmermann & Briegei, 1993; Lucas & Romoser, 2001). Cognitive abilities of mosquitoes in their pre-imaginal stages have been barely studied. Recently, Baglan *et al.* (2017) reported habituation leaving a mnesic trace lasting for one hour in mosquito larvae of *A. aegypti*. Analogously to the work performed by Brunner & Maldonado (1988) in the crab *Neohelice granulata* (*Chasmagnathus granulatus*), Baglan *et al.* (2017) quantified the reduction of the escape response to the shadow projected by an object moving over the surface of the water (named by the authors as "visual danger stimulus") subsequent to repeated presentations.

According to Chilaka *et al.* (2012), it is particularly important to study learning and memory in mosquitoes and other insects that transmit human diseases because their behaviour determines their success as disease vectors (McCall & Kelly, 2002; Alonso & Schuck-Paim, 2006; Bouyer *et al.*, 2007; Vinauger *et al.*, 2014, 2016). Therefore, behavioural studies in mosquitoes must take into account all the developmental stages, since larval performances have an impact on their adult success (McCall & Eaton, 2001; Kaur *et al.*, 2003). So far, studies on cognitive abilities in pre-imaginal stages remain scarce, as mentioned above.

Habituation is traditionally understood as a non-associative form of learning. However, during habituation, animals can integrate other stimuli constantly present in their environment. Those stimuli altogether are known as the *context*, *i.e.* the surrounding conditions where learning takes place (Myers & Gluck, 1994). Contextual stimuli can be associated with habituation stimuli. When such an association exists, it determines the contextual specificity of habituation. Context-dependency in habituation was demonstrated in the nematode *C. elegans* (Rankin *et al.*, 1990; Nirit & van der Kooy, 2000; Rankin, 2000) and in the sea-hare *Aplysia californica* (Pinsker *et al.*, 1970; Carew & Kandel, 1973). In the crab *N. granulata*, it was shown that when the context was changed, habituation to a shadow passing over the crab was reversed (Hermitte *et al.*, 1999).

The fact that context-dependency of habituation has been observed in diverse organisms let us hypothesise that this is a generalised phenomenon. Consequently, we asked whether habituation in the larvae of *A. aegypti* was context-dependent as well.

In the present work, we studied if mosquito larvae were able to associate visual contextual stimuli to the stimulus inducing habituation. To that end, mosquito larvae were individually trained in a Petri dish whose bottom presented a set visual pattern (the *context*). Once habituation was induced, the *context* was changed and the shadow stimulus was presented again. Context-dependency of habituation would be evinced, if the larvae performed the escape response.

Materials and Methods

Experimental subjects.

Fourth-instar larvae of *Aedes aegypti* Bora strain were used in this work. Mosquitoes were reared in a climate-controlled room at 25°C with 12:12 L:D cycle. Larvae were obtained from eggs provided by the Laboratoire d'Insectes Nuisibles (MIVEGEC-IRD, Montpellier, France). Eggs were put in small plastic containers (l: 12 cm; w: 6 cm; h: 6 cm) with dechlorinated tap water. Larvae were fed with shrimp food (JBL Novo Prawn, Neuhofen, Germany; www.jbl.de). Under those conditions, larvae reached their fourth instar after five days and moulted into pupae five days later, *i.e.* it took around 10 days from hatching to pupal moult. In order to avoid using pharate pupae, we did not use larvae older than eight days. All the animals were reared and treated according to the rules and regulations on ethics applied in the European Union.

Apparatus

The protocol used in the experiments was adapted from Baglan *et al.* (2017). The experiments were performed in a different room at 24 ± 1°C. Each larva was individually placed into a Petri dish (3 cm diameter and 1.2 cm height) filled with dechlorinated tap water. Each Petri dish was placed on a table directly under a spot light. Under each Petri dish a square card-board piece was located in order to determine the visual context. The card-board piece determining the context was painted with one of the following three patterns: 100% white, 100% black or striped in black and white (each stripe was 1 cm wide; black and white covered 50% of the surface, each). The Petri dishes were visually isolated from each other by opaque walls (30 cm in height and width). To avoid any disruption caused by vibrations, each Petri dish was placed on a foam block.

The visual stimulus was presented by a mechanical arm, namely, a wooden stick of 18 cm long, with a square piece of card-board of 3 x 3 cm at the end. The square card-board projected a shadow stimulus to be perceived by the naïve larva, this signal might be similar to the visual stimulus performed by an aerial predator in nature. Consequently, the naïve larva performed the escape response. We used a servomotor associated with a control board («Arduino Uno»; http://www.arduino.cc) to automatically deliver the *habituation stimulus*. The angular speed of the stimulus 315.8°/s at 4.8 V.

Habituation protocol

The protocol of habituation included three phases: *acclimatation*, *training* and *test*. During *acclimatation*, each larva was deposited in a Petri dish under the spot light and rested during 30 minutes. Over the course of the *training* phase the mechanical arm projected its shadow on the Petri dish during 1.5 s, with an inter-trial interval of five minutes. *Training* had a total duration of one hour and 15 min. The visual stimulus was displayed 15 times, *i.e.* 15 trials. In every trial, the response of the larva was recorded, *i.e.* escape response = 1; no response = 0. The escape response is evinced by fast movements of the abdomen, letting the animal dive (see the video in Baglan *et al.*, 2017). A decrease in the escape response over the course of the training phase would indicate habituation (see Baglan *et al.*, 2017). Once the *training* phase was completed, the Petri dish was very gently raised to avoid any disturbance that could provoke dishabituation (Baglan *et al.*, 2017). The card-board piece underneath (the *context*) was taken away and replaced either by another one or put back, in experimental or control groups, respectively. Once the context was either replaced or put back, larvae were left resting during 10 minutes. Subsequently the test was performed. The *test* phase consisted in one presentation of the visual stimulus.

Experimental design

Three different types of background (white, black or striped) were used as *context* (*i.e.* the stimulus that was present during the whole *training* phase; Nadel & Willmer, 1980), for both *training* and *test.* All possible combinations represented nine independent experimental groups, each group included 30 replicates. In total, 270 individuals were included (Figure 1).

Data analyses.

The data analyses were performed using a Bayesian model selection procedure on a series of mathematical models describing the habituation process, starting with the simplest model and increasingly adding complexity until the information criterion used worsened or the full model with all the parameters was reached.

Learning model

A series of Hidden Markovian models (Eddy, 2004), based on the Power-Law of Practice (Snoddy, 1926), was used to explain the dynamics of learning. Our explanations were based on the experimental data. According to this approach, learning does not occur at a constant speed but is slowed down over the course of the habituation trials. In the beginning, learning occurs faster, however, further improvements are more difficult. Therefore, Power-Law models propose that the learning decreases linearly with the logarithm of the number of practice trials taken, thus producing an exponential decay, in which the response tends asymptotically towards a minimum value (generally zero). In this case, the escape response is expected to decrease as the experimental subject is subjected to successive tests until it approaches a minimum baseline value (See “Results” for details).

The model we applied is well known and it was widely tested in human learning (See Fitts & Posner, 1964; Anderson, 1982; Card *et al.*, 2017). More recently, Evans *et al.* (2018) developed Bayesian versions of this model.

The basic structure of the model is:

$$R_t = (1-h)\ R_{(t-1)}$$

$$R_e = (1-R_a)\ R_t + R_a \quad \text{(Eq 1)}$$

in which habituation is expressed as a response (R_t), which decreases exponentially at a rate h towards zero. As the final response can be greater than zero, then the response R_t is rescaled so that instead of tending asymptotically to zero, it tends towards a value called R_a, which would be the average response of the experimental subjects after a infinite number of trials, the rescaled value is called R_e.

Once the tests were finished, the habituation could decrease or not, according to a value called d during the test.

The full development of the model is in the Appendix 1.

Statistical methods

The main objective of the analyses was to define whether or not the model parameters differed among treatments (test settings, and their combinations), and to what degree. Once we defined which parameters were specific to the treatments and which parameters were general to the learning model, the values of the parameters were calculated.

The model describing learning is non linear. Hence, the parameters could interact with each other in many possible ways. As a consequence, we used a process of selection of models through information indices to obtain an optimal model instead of simple statistical tests of the null hypothesis. These parameters would be treatment-specific only in the event that a given parameter varied between treatments, and would be common to all in case the parameter did not vary between treatments.

Consequently, the data were analysed using a stepwise model selection procedure under a full Bayesian approach. We proposed a series of explanatory models which were generated from the null model becoming more complex by adding parameters on each step, thus increasing complexity. The development of these models continued until the model complexity exceeded the increase in complexity caused in the model by adding a new parameter. The deviance information criterion (DIC; Gelman *et al.*, 2004) was used as a decision rule for accepting/rejecting the proposed models. When a newly more complex model was rejected, because the DIC increased, the procedure stopped, and we kept the last selected model as the explanatory model.

The distribution of parameters of the models was calculated using the Markov Chain Montecarlo (MCMC) algorithm with the pymc module for stochastic modelling in python version 2.3.8 (Patil *et al.*, 2010).

Non-informative uniform *a priori* distributions between 0 and 1 were used for all the parameters, except the autocorrelation coefficient, which was between -1 and 1. A total of 200,000 iterations of the MCMC were used, of which the first 100,000 were discarded as a *burn-in*, and the remaining were used to estimate the *a posteriori* distributions.

Using a Bayesian approach provides a more straightforward way to fit and select among a great number of models, unlike frequentist statistical methods, and the same approach had already been used by ecologists in animal behaviour studies (Ellison, 2004; McNamara *et al.*, 2006; Pietrantuono *et al.*, 2015, 2017).

Additionally, the generalised coefficient of determination (*GCD*) for binary data, according to Cox & Snell (1989) and Magee (1990), was calculated as a measure of the fitness of each of the proposed models to the data of the experiments.

Results

Resulting model of Aedes aegypti habituation

As a result a total of 31 models (plus null model) were developed. Through the model selection process, we obtained a model that, according to the used information criteria (minimum DIC), is optimal in terms of explanatory power and complexity (model # 18, Table A1, Appendix 2). The resulting model contains six parameters (h, d_c, R_{aw}, R_{ab}, R_{as}, *and* φ), detailed in Table A1, and its explanatory power in terms of *GCD* is 0.9611. This means that this model is capable of explaining 96% of the variance of the study data. As explained above, all parameters that differed among *test* contexts, testing or their interactions, are included in the model as separate parameters (with a subscript j), while the parameters that do not differ were replaced by a single common parameter for all treatments.

The model itself consists of the one described in equation 7 of Appendix 1 with one parameter R_a (*asymptotic response*) depending on the context (one parameter per context, *w* (white), *b* (black), *s* (striped), resulting in three asymptotic responses: R_{aw}, R_{ab}, R_{as}), the increase in response occurred only in the case of change of the context, but not of the context itself, so it is called d_c (here the subscript *c* is used to denote the change of context) and the parameters *h* (the *habituation rate*) and φ (the *autoregressive coefficient*) independent of any context. In this way the final model of learning by habituation for *Aedes aegypti* was:

$$R_{tj} = \delta_j (1- h)^t + \varphi (O_{i(t-1)} - R_{oi(t-1)}) + (1- \delta_j)(d_c (j \neq k)) \text{ (Eq 2)}$$

where in equation 2 of the appendix, the response decays exponentially to R_a instead of zero as the conditioning trials are performed, so the final observed response is R_{etj}

$$R_{etj} = (1-R_{aj}) R_{tj} + R_{aj}$$

being j and *k* the trial and test context respectively, both can take the *w*, *b*, or *s* values, δ is a Kronecker delta which indicates that the insect is in trial ($\delta = 1$) or test ($\delta = 0$) context, and O_i is the observed response for the individual *i* at trial *t*.

The parameters calculated for this model exposed in Table 1 show that, on the one hand, the habituation rate was the same for all the contexts, whilst, on the other hand, the asymptotic response depended on the context, being similar between white, and striped background, but higher in black. As explained in *Materials and methods*, the asymptotic response had an inverse relationship with habituation, so the white and striped backgrounds favoured habituation in the long term. As can be seen in Figure 2, the final responses in white and striped backgrounds were similar, and the response was the greatest in black backgrounds, with similarly sloped curves in all the cases.

Finally, autocorrelation was strong, since the autoregressive coefficient (φ) was estimated to be 0.3 the (Table A1, Appendix 2) and could twist the results in unpredictable ways if not taken into account.

Response levels in training *and* test *phases*

The results evinced that the insects included in this study had a Power-Law type learning curve, in which the response decreased to a level which is higher than zero, but lower than the initial response level. Over the course of the 15 training trials, the response to the visual stimulus decreased in all the cases (Figure 2). Therefore, habituation was observed independently of the visual background (*context*).

Some parameters of the learning curves were shared across all the contexts, while other were context-specific (Table 1). All the backgrounds shared the same habituation rate (h), but differed in their asymptotic responses (R_a), as a consequence the speed of habituation was similar regardless of the background (visual *context*).

The parameter d (*response-increase*), only present (*i.e.*: different from zero) in the case of background shift, means that in the *test* phase, the insects retained the same R_a level of escape/response level as at the end of the *training* phase in case of using the same background, as it is possible to observe in Figure 2 (a, e and i). In case of changing the background between *training* and *test*, the response increased. The evinced increase in response may be attributable to the background shift (Figure 2 b, c, d f, g and h).

Discussion

In all the visual backgrounds applied in the present work, mosquito larvae *Aedes aegypti* could be habituated to a visual stimulus. In all the cases, the decrease in response due to habituation did not depend on the visual background (*context*), which was evinced by the fact that the slopes among all the learning curves did not differ. However, habituation level was the lowest, when larvae were trained in the black visual *context*. In all the cases, changing the visual *context* between the *training* and the *test* phases induced an increase in the response in the *test*, thus proving that habituation was context-dependent.

As mentioned above, larvae could be habituated in all the contexts. At the same time, we observed that, over the course of the *training* phase, the decrease in escape response occurred at the same rate in the first five trials, *i.e.* the slopes of the acquisition curves did not differ among each other. In subsequent trials, behaviour differed according to the visual context. When larvae were trained in a black context, the slope decreased from the 6th trial on and the response reached its asymptote at 10% greater response level than in the cases in which larvae were trained in either a striped or a white *context*. Therefore, habituation performances depended on the visual context. In particular, the larvae exhibited their best learning performances when trained in lighter visual contexts. In those cases, the contrast between the shadow of the moving cardboard square was greater when projected on white than on black surfaces. The lower habituation level observed on the black *context* could be due to the contrast between the shadow and the background being lower than in the other two *contexts*. Quantifying the effects of contrast on habituation level is a question that deserves future experiments. For example, larvae could be trained on different backgrounds of a solid greyscale or striped backgrounds varying the width of the stripes or the white/black area ratio.

In our study, mosquito larvae habituated to the visual stimulus on a white background no longer responded to the visual stimulus when tested on a white background, whilst they responded to the same stimulus when tested on a black background. Conversely, the same stimulus presented on a different visual background, namely, a different *context*, was perceived as a novel stimulus inducing larvae to escape. Context-specificity let us understand that there must have been some kind of associative component of habituation between context and the visual stimulus, as had been observed in previous work in crabs (Tomsic *et al.*, 1998; Hermitte *et al.*,1999; Pereyra *et al.*, 2000).

Thanks to the use of Markovian models combined with Bayesian statistics, it was possible to make an accurate description of the mosquito learning curve. Likewise, it was possible to formulate parameters with great biological significance, improving what has been possible so far using generic statistical models such as those that are most commonly used to analyse habituation experiments. In this work, we calculated the rate, and the final habituation value, the increase in response produced at the end of the training procedure, and its dependency on the context, all corrected by autocorrelation, using an autoregressive (AR) term. Therefore, we suggest the use of this methodological approach to analyse this type of experiments in the future, which significantly increases the amount of information in a study, while maintaining the same design and experimental effort, using the same laboratory equipment.

Our results give some contribution to the so far scarcely explored learning abilities of the mosquito larvae of *Aedes aegypti*, in particular, the ability to associate a constantly present visual stimulus (the *context*) to moving object. Are mosquito larvae capable of associative learning? The question remains open.

Comprehensive understanding of the pre-imaginal behaviour of mosquitoes, in particular, the behavioural plasticity, will certainly allow us better know about their adaptability to different environments. An important piece of the jig-saw puzzle of integrative pest control and environmental conservation.

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Author contributions

ALP, MBA and FG conceived and designed the experiments; ALP and MBA performed the experiments; OB analysed the data; ALP, MBA, OB and FG wrote the paper.

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Appendix 1

Model description

A series of hidden Markovian Models (Eddy, 2004), were proposed to explain habituation. The hidden Markovian model assumes that habituation is a stochastic process in which, as in any Markovian process, future states depend only on the current state. Here, the state variable R (the expected response, *i.e.*: the proportion of times in which a given animal will respond to the presented stimulus) cannot be observed directly at an individual level (so the model is hidden), but can be inferred after observing the response of the experimental animal after each test. If R is a proportion, it is restricted to values between zero and one, therefore R as a function of time was modelled as a Power-Law learning curve as follows:

$$R_t = (1-h)\, R_t-1 \quad \text{(Eq. 1)}$$

where R_t is the expected response at trial t, and h is the habituation rate, with h being $0 \leq h \leq 1$, so if h is equal to zero, there is no habituation ($R_t = R_t-1$), and if h is one, the maximum habituation is reached after only one trial ($R_t = 0$), independently of the value of R_t-1. In this model, R decays exponentially to zero after an infinite number of trials, however it is possible that the asymptotic response will be different from zero, after a large number of trials some animals still will be responding positively to the stimulus, so we called R_a to that asymptotic response (a lower asymptotic response means higher habituation). Now the equation 1 is modified as follows:

$$R_e = (1-R_a)\, R_t + R_a \quad \text{(Eq. 2)}$$

where R_e is the expected response which is R_t multiplied by $1-R_a$ plus R_a, so the response decays exponentially to R_a instead of zero as the conditioning trials are performed.

Also there is increase in response after the *training* phase finished. The observed increase in response is also assumed to be a function with exponential decay, but now in reverse direction, so R_t increase asymptotically to one. Therefore, the model is modified as:

$$R_t = \delta\, (1-h)\, R_{t-1} + (1-\delta)\, (1 - (1-d)\, R_{t-1}) + \varphi\, (O_{i(t-1)} - R_{e(t-1)}) \quad \text{(Eq. 3)}$$

where δ is a Kronecker delta which is one if the animal is in a conditioning trial and zero otherwise, and d is the response in the test. So, the first term (exponential decay in response) is active during the conditioning trials, and the other is zero, whereas during the *test* phase the first term is zero, and the second is active, giving an exponential increase. Finally, to compensate for autocorrelation in the response of the same individual used in the trials, an autoregressive term (AR) was added. This term attempt to correct for the increase (or decrease) in the expected response, after the experimental animal gave a positive or negative response to the stimulus.

$$R_t = \delta\,(1-h)\,R_{t-1} + (1-\delta)\,(1-(1-d)\,R_{t-1}) + \varphi\,(O_{i(t-1)} - R_{e(t-1)}) \quad \text{(Eq. 4)}$$

where φ is the autocorrelation coefficient, $Oi(t-1)$ is the observed response of the animal i in the previous trial, and $R_e(t-1)$ is the expected response.

Then, it was possible to simplify, since all the animals had the same initial conditions at $t = 0$, with $R_t = 0 = 1$, and that only one test trial was performed. The full model for any trial was set as follows:

$$R_t = \delta\,(1-h)^t + \varphi\,(O_{i(t-1)} - R_{oi(t-1)}) + (1-\delta)\,d \quad \text{(Eq. 5)}$$

If the response of the experimental animal were context dependent, the equation 5 could be modified as follows:

$$R_{tj} = \delta\,(1-h_j)^t + \varphi\,(O_{i(t-1)} - R_{oi(t-1)}) + (1-\delta_j)\,d \quad \text{(Eq. 6)}$$

where as in equation 2, the response decays exponentially to R_a instead of zero as the conditioning trials are performed, so the final observed response is R_{etj}

$$R_{etj} = (1-R_{aj})\,R_{tj} + R_{aj}$$

where the sub index j corresponds to the context in which the trial was performed. Intermediate models between equations 1 and 2 were possible, with some of the parameters p, h and d being context dependent. Furthermore, under this experimental design, the *test* phase could be performed in a different context from the *training* phase, so the equation 2 could be modified to add another sub index called k which is the context in which the trial stage was performed

Then, the variable d became dependent in two different contexts j and k. If j and k represented the total number of contexts used in *training* and *test* phases respectively, the d variable comprehended j parameters, if it depended on habituation context, k parameters if they only depended on test phase context, and jxk parameters, if they depended on both contexts. Finally, there was the possibility that *response-increase* did not depend on the context itself, but on the change of context:

$$R_{tj} = \delta\,(1 - h_j)^t + \varphi\,(O_{i\,(t-1)} - R_{oi\,(t-1)}) + (1 - \delta_j)\,(d_e\,(j=k) + d_c\,(j \neq k)) \quad \text{(Eq. 7)}$$

where the asymptote is higher than zero, as in equation 2, so the final observed response is R_{etj}

$$R_{etj} = (1-R_{aj})\,R_{tj} + R_{aj}$$

Thus, there were two possible *response-increase* parameters, one (de) which corresponded to the expected response-increase, if the test context were the same as the conditioning context ($j=k$), and a second ($j \neq k$) which corresponded to the expected increase in response if the test context were different than the conditioning context (dc). See Figure A1.

Appendix2

Model selection procedure, by means of the deviation information criterion (DIC).

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Figuress

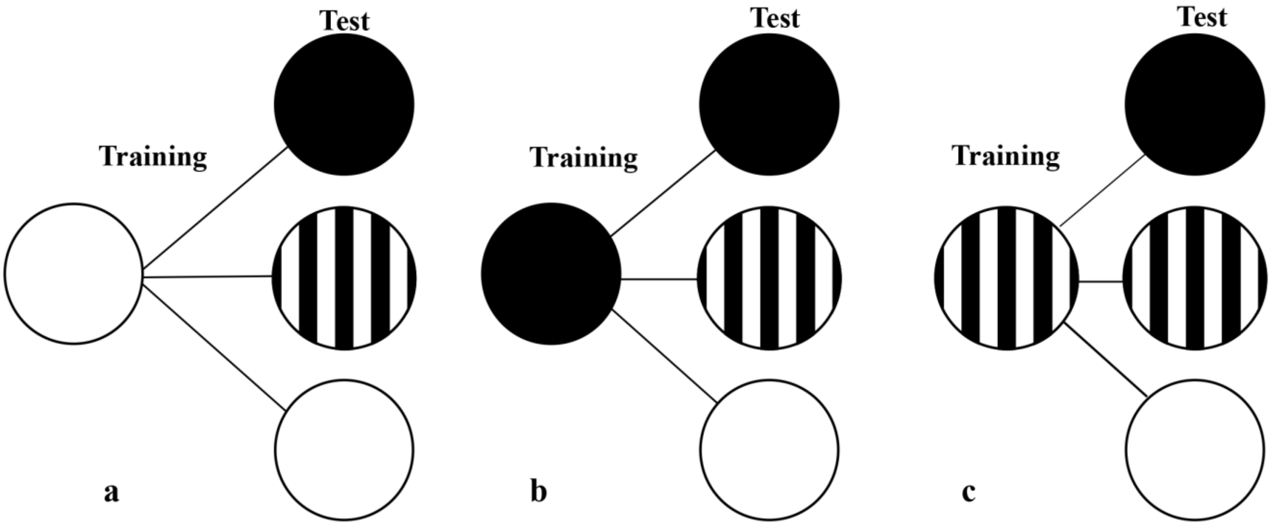

Figure 1. Experimental design. Three backgrounds were used as *context* in the *training* and in the *test* phases: a: white; b: black; c: striped. Each visual background was replaced between the end of the *training* phase and the *test* phase. The visual background in the *test* was shifted in the experimental groups, *i.e.* nine treatments were performed. In each group, n = 30.

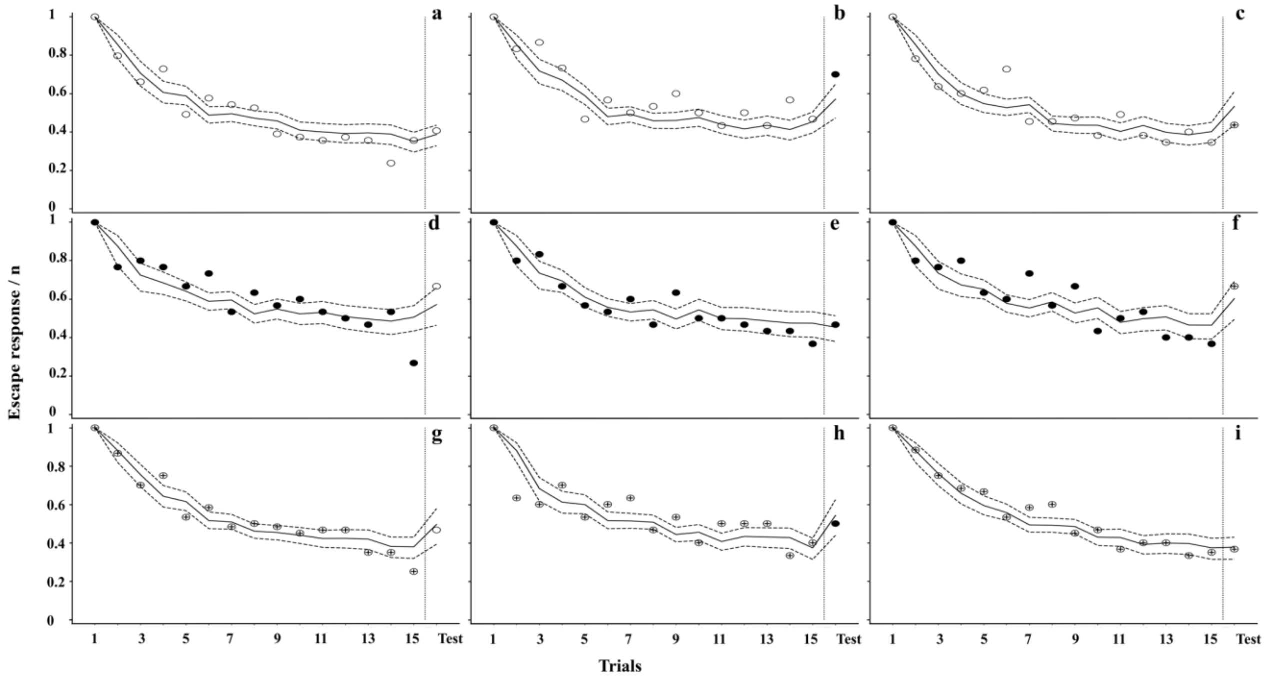

Figure 2. Response levels in *training* and *test* phases. Response level expressed as the number of larvae performing escape responses on the presentation of the visual stimulus out of the total number of assayed individuals. The abscise axis indicates the 15 *training* trials plus the *test*. White, black or stripped backgrounds are indicated by white, black or crossed circles, respectively. Black lines indicate the *a posteriori* estimated mean responses and dotted lines indicate 95% credibility interval. The vertical line separates *training* from *test*. In each group, n = 30.

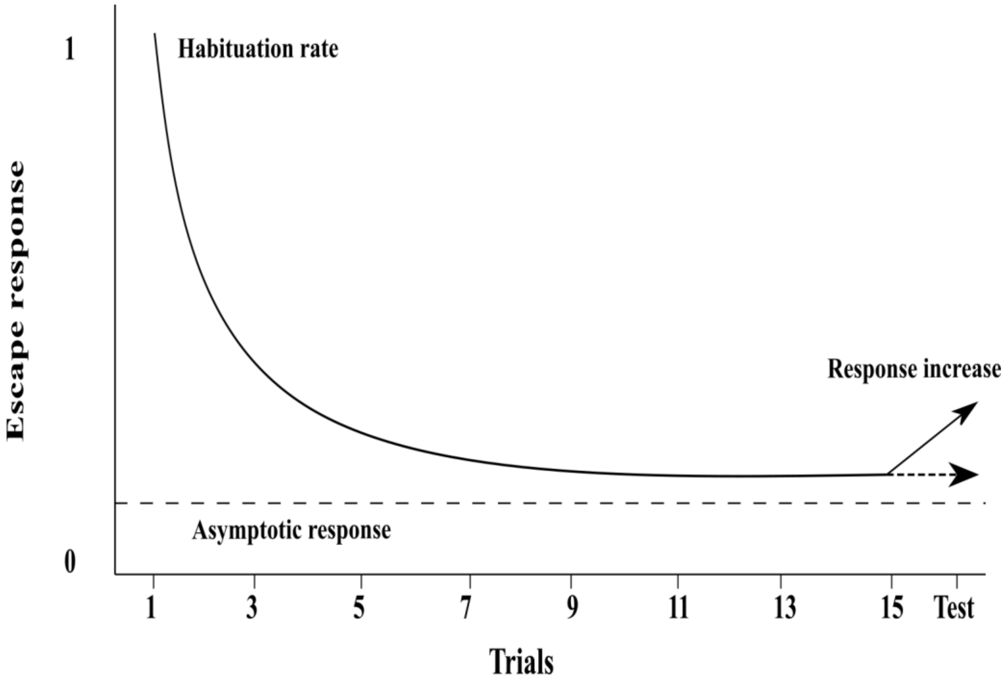

Figure A1. Conceptual Model of Habituation Learning Curve. Graph showing the habituation curve proposed in this study. Habituation is assumed to produce a decreasing mean response that decays exponentially at a given rate until the trials are completed. The initial response is one, and it decays to an asymptotic value higher than zero, shown in a dotted line.

Table 1. Estimated parameters of the selected model. Values of each parameter from the selected model and its 95% credibility intervals.

Parameter	**Symbol**	**Context**	**Mean Estimation and 95% Credibility Interval of response at *Training* phase**
Habituation rate	h	All	0.306 [0.266 - 0.351]
Response increase	d_c	Different	0.149 [0.098 - 0.200]
Asymptotic response	Ra_w	White	0.270 [0.240 - 0.296]
	Ra_b	Black	0.324 [0.293 - 0.358]
	Ra_s	Striped	0.278 [0.248 - 0.305]
Autoregressive coefficient	φ	All	0.311 [0.283 - 0.338]

Table A1. Models proposed to explain the behaviour of the assayed larvae. Models proposed in the Bayesian selection process, its parameters and values of the deviation information criterion (DIC), and generalised coefficient of determination for logistic distribution (GCD), according to Cox & Snell (1989) and Magee (1990). Text in bold indicates the selected model with lowest value of DIC. The null model does not have a coefficient of determination, because it is used to calculate this coefficient for the other models. The model terms are abbreviated as follows, habituation rate is *Hr* (*h* mathematical symbol), asymptotic response is *Ra* (*Ra* mathematical symbol), and the autoregressive term is AR (φ).

Model N°	**Model**	**Variables**	**Variables dependent on context**	**N° of parameters**	**DIC values**	**GCD**
0	Null model (*Hr = 0*)	R_a	-	1	7967.14	-
1	*Hr* (*Ra = 0*)	h	-	1	7786.11	0.4885
2	*Hr* + *Ra*	h, R_a	-	2	7607.97	0.7375
3	*Hr* depending on context (*Ra = 0*)	h	h	3	7767.95	0.5288
4	*Hr* depending on context + *Ra*	h, R_a	h	4	7594.21	0.7543
5	Model 3 + *Ra* depending on context	h, R_a	R_a	4	7590.99	0.7572
6	Model 4 + *Ra* depending on context	h, R_a	h, R_a	6	7593.31	0.7587
7	Pure *Ra* model (*h = 0*)	R_a, φ	-	2	7387.26	0.8841
8	*Hr* + *Ra* (*Ra = 0*)	h, φ	-	2	7244.82	0.9316
9	Model 3 + AR	h, R_a, φ	-	3	7128.27	0.9559
10	Model 4 + AR	h, R_a, φ	h	5	7127.37	0.9567
11	Model 5 + AR	h, R_a, φ	R_a	5	7121.10	0.9577
12	Model 6 + AR	h, R_a, φ	h, R_a	7	7122.59	0.9581
13	Model 9 + response-increase	h, R_a, φ, d	-	4	7116.83	0.9581
14	Model 10 + response-increase	h, R_a, φ, d	h	6	7114.82	0.9590

15	Model 11 + response-increase	h, R_a, φ, d	R_a	6	7109.93	0.9597
16	Model 12 + response-increase	h, R_a, φ, d	h, R_a	8	7111.68	0.9601
17	Model 14 + response-increase only when changing context $j \neq k$	h, R_a, φ, d	$H, d(j \neq k)$	6	7105.73	0.9603
18	**Model 15 + response-increase only when changing context $j \neq k$**	**h, R_a, φ, d**	**$R_a, d(j \neq k)$**	**6**	**7100.52***	**0.9611**
19	Model 16 + response-increase only when changing context $j \neq k$	h, R_a, φ, d	$h, R_a, d(j \neq k)$	8	7103.38	0.9613
20	Model 14 + response-increase depending on same contex ($j = k$) or change of context ($j \neq k$)	h, R_a, φ, d	$h, d(j \neq k)(j=k)$	7	7107.45	0.9604
21	Model 15 + response-increase depending on same contex ($j=k$) or change of context ($j{\neq}k$)	h, R_a, φ, d	$R_a, d(j \neq k)(j=k)$	7	7102.39	0.9611
22	Model 16 + response-increase depending on same contex ($j=k$) or change of context ($j \neq k$)	h, R_a, φ, d	$h, R_a, d(j \neq k)(j=k)$	9	7105.18	0.9613
23	Model 14 + response-increase depending on habituation context (j)	h, R_a, φ, d	$h, d(j)$	8	7111.62	0.9601
24	Model 15 + response-increase depending on habituation context (j)	h, R_a, φ, d	$R_a, d(j)$	8	7109.29	0.9604
25	Model 16 + response-increase depending on habituation	h, R_a, φ, d	$h, R_a, d(j)$	10	7112.76	0.9605

	context (*j*)					
26	Model 14 + response-increase depending on test context (*j*)	h, R_a, φ ,d	$h, d(k)$	8	7116.30	0.9594
27	Model 15 + response-increase depending on test context (*j*)	h, R_a, φ, d	$R_a, d(k)$	8	7111.59	0.9601
28	Model 16 + response-increase depending on test context (*k*)	h, R_a, φ, d	$h, R_a, d(k)$	10	7114.02	0.9603
29	Model 14 + response-increase the combination of both habituation and test context (*j*, *k*)	h, R_a, φ, d	$h, d(j, k)$	14	7110.51	0.9620
30	Model 15 + response-increase the combination of both habituation and test context (*j*, *k*)	h, R_a, φ, d	$R_a, d(j, k)$	14	7107.77	09623
31	Model 16 + response-increase the combination of both habituation and test context (*j*, *k*)	h, R_a, φ, d	$h, R_a, d(j, k)$	16	7110.34	0.9625