Stimulus integration in Tenebrio larvae: an easy laboratory study (or how to show Euclidean larvae)

Xavier Espadaler

Through a simple analysis of an immediate response to light, it is possible to show stimulus integration in Tenebrio larvae and introduce circular statistics.

Introduction

Sensory organs are finely tuned for the detection of relevant stimuli. Not all environmental factors—e.g., vegetation properties, temperature, humidity—are significant to all animals. Each species has a particular array of sensory mechanisms. When in action, they provide individuals with perceptual windows. Receptors filter those stimuli that have some kind of meaning for the animal (Slater, 1985). So, each species has its own significant world, its ‘Umwelt’.

Both stimulus quality and quantity are subjected to this scheme of selective filtering. In this article it is shown through an easy method how an organism, via a generalized response—negative phototaxis—can evaluate a stimulus quantitatively. I have used this experiment for many years in my laboratory course on animal behaviour. Larvae behave as if they were fluent in trigonometry!

Materials

The beetle Tenebrio molitor L. (Coleoptera, Tenebrionidae) is easily cultured, and provides material for many general observations (protozoa gregarinidae in the gut; metamorphosis; sexual behaviour and mate guarding). Tenebrio larvae have an advantage over fly larvae, especially as they are easier to keep and the larval stage is of far greater duration. Active larvae are chosen and marked with rapidly drying paint to identify individuals. Inactive larvae are in the process of moulting or pupating; they remain motionless, slightly curved, and look softer when touched. They are not responsive to stimuli and should not be used. Two lamps, with pearl light bulbs (100 W, 40 W). Polar co-ordinates, 20 cm diameter, divided into 36 numbered sectors. A small rectangular cardboard box (e.g. 1 x 1 x 4 cm); alternatively, a V-shaped piece of light card. A sheet (figure 1) with ad hoc tables to note individual scores.

Methods

The laboratory should be dark or have a very dim light. Each pair of students (group) has six larvae. The polar co-ordinates and sectors should be marked, to form the arena out on a flat surface. A sheet of cardboard on a plastic surface table with suitable markers could be used. An arbitrary ‘north’ direction is chosen as in front of the observer. Two different tests are run.

Test 1
One lamp (100 W) is placed at an angle of 45° to this front direction (figure 2). A larva is picked up gently with forceps and put inside the small rectangular box (or onto the V-shaped card), and the box placed on
the centre of the co-ordinates; with a light tap the larva is gently released so that it is heading towards the ‘north’ on the co-ordinates, and simultaneously the light is turned on. Larvae are negatively phototropic.

Observe and note: a) side (right–left) of first head movement; b) sector traversed by the larva when fleeing from the light. All six larvae are scored, and the process is repeated five times. This test usually takes about an hour.

**Test 2**
Both lamps are involved (figure 2); 100 W right, 40 W left. Run the test with the same six different larvae. Note the scores as in test 1.

**Figure 2** Laboratory settings for test 1: a single lamp (100 W) at 45° on the right. For test 2, two lights are needed: 100 W on the right, and 40 W on the left. The larva is positioned heading ‘north’.

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**Figure 1** A table to indicate individual scores for six larvae through five runs.

**Results**

Inspection of the results from individual groups should highlight any gross individual variation between larvae; if so, a fruitful discussion can follow on the convenience—or not—of including anomalous individuals in the analysis and the significance of such variation. Usually, we decide to discard outliers.

If all groups of students have the same orientation in the laboratory and the lamps are similarly positioned, viz. 100 W right, 40 W left, all the data can be pooled. The same orientation of all groups reduces another variable, as the possibility of some kind of magnetic response has been suggested for *Tenebrio* adults (Arendse, 1980). Table 1 shows actual results of pooled data from 10 student groups. They can be graphed on both polar and cartesian co-ordinates (figures 3 and 4). Table 2 shows the corresponding right vs left first head movement in both tests.

**General comments**

The tests, as run, are open to many criticisms; they are intended to provide material for discussion about the investigation. The more common criticisms are as follows:

- Light bulbs are also a source of heat. The two stimuli (light, heat) may have been acting on the release of the behavioural response. We are not sure if they have additive properties, and propose some experimental tests to discriminate between both factors. Test as usual vs test with a heat filter (e.g. two thick glasses separated by 1 cm); are the results similar? Does velocity of the escape response show any variation?
- Light bulbs are not unidirectional. They make a wide angle of light; this could account for the
rather broad distribution of sector scores. Simple solutions such as a hole (1–2 cm diameter) on dark cardboard in front of the light are often suggested as a way to circumvent this source of variation.

- Polar co-ordinates are not changed after each run. The first larva could have left some kind of chemical signal, and other larvae simply follow the trail. The solution is easy; but spare paper and save a tree.
- Other variables such as sex, the feeding state of larvae, and the age of larvae may also be discussed; also, the table top may not be level. Adults are positively phototactic after desiccation but negatively phototactic in normal conditions.

Data analysis

With 10 groups, some 300 scores for each test are obtained. Two levels of analysis can be envisaged.

First level analysis

A short exercise in trigonometry should enable one to predict the exact shift anticipated if larvae could evaluate quantity (intensity) of light stimuli (figure 5). This is detected through a comparison of results of tests 1 and 2. With a 100 W light bulb on the right and a 40 W on the left, the precise expected shift is 21.8°, assuming that the 40 W bulb emits 2/5 times as much light as the 100 W. The data obtained are circular in nature and such data have peculiar properties; an entire field of circular statistics has been produced to analyse orientations, animal movement paths, flight directions, etc. (Batschelet, 1981; Mardia, 1972). A good, brief summary is given by Cain (1989). As a measure of central tendency, the median direction or median angle is a good approximation to the mean vector direction when the distribution of circular data is unimodal and symmetric; judge from visual inspec-

![Figure 3](image-url)
tion of the data in cartesian co-ordinates or, better, through skewness measures.

If we assume for each sector a value of 5°, 15°, 25°, 35° and so on, the mean angle ± angular deviation for

test 1 (table 1) is $231.7° ± 38.9°$; the length of the mean vector is the most frequently used measure of location, it takes a value from 0 to 1. For test 1, the mean vector is 0.769. For test 2, the mean angle is $204.1° ± 39.7°$, and the mean vector length is 0.759.

When animals are clearly orientated, as happens here, to test whether a difference exists between a previously expected direction and the experimental result, confidence intervals for the mean angle are used (Batschelet, 1981). With our sample of 279 scores and a 95 per cent confidence coefficient, the confidence limits of the mean angle are $204.1° ± 4°$ (Batschelet, 1981). For test 2, if there was not stimulus

<table>
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<tr>
<th>Table 2</th>
<th>Right vs left first head movement of Tenebrio molitor larvae when receiving the light. Pooled data from 10 student groups</th>
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<tr>
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<td>Right</td>
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<tr>
<td>100 W right</td>
<td>52</td>
</tr>
<tr>
<td>100 W right, 40 W left</td>
<td>83</td>
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integration, the expected mean direction would be 180°; since this value lies outside the confidence interval, we conclude that the two angles—180° vs 204.1°—differ.

If larvae could evaluate ‘quantity’ in stimuli, then the medians of tests 1 and 2, under the conditions stated above, should differ in 21.8° and, so, three sectors. They do. However, one must remember that these subjects can form no real visual image, and are limited to simple kinotactic behaviour.

A more rigorous approach would be to control for independence of measures and not commit the pooling fallacy (Machlis, Dodd, and Fentress, 1985). On both tests, with circular statistics, we must find the mean direction of the six runs for each larva (the independent measure) and then the mean direction of all individual mean directions. Then, this single value can be compared for both tests. The chi-squared test of right–left frequency turns when two lights are present confirms the results obtained when a single source of light is present (table 2). Data, though, are not so sharply biased and show that a new factor (40 W left light lamp) is in action.

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References


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