STUDIES ON THE LIGHT RESPONSE OF THE AMMOCOETE LARVA OF LAMPETRA PLANERI (BLOCH) AND LAMPETRA FLUVIATILIS (LINN.).

by

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1. **INTRODUCTION.**

The behaviour of an animal may be modified by changes in its external environment. The environment can be analysed in this respect into a number of stimuli which by virtue of their nature and intensity activate the various sense organs of the animal to different degrees. The present work is concerned with the way in which differential activity in more than one sense organ can produce a change in behaviour. Its aim is to investigate a modification of the response to one stimulus by the introduction of a second stimulus and to determine a relation between the modified response and the intensities of the two stimuli.

The work is an attempt to relate behaviour to the physical properties of the environment that activate the sense organs. Its approach is more likely to lead to an analysis of behaviour in terms of the physiology of the nervous system than purely descriptive work which is analysed in reference to models, similar to those used in psychology.

The response to light of the ammocoete larva of *Lampetra planeri* (Bloch) was used for this work. Young (1935) showed that the response is a random movement unrelated to the direction of the light and that a delay, referred to as the reaction time,
occurs between the onset of illumination and the beginning of movement. Steven (1950) found that a constant feature of the response is the inverse relation between the reaction time and the light intensity. Young (1935) also showed that the whole body of the ammocoete is sensitive to light, the region of greatest sensitivity lying in the dorsal fluke of the tail; the photoreceptors concerned in the response are innervated by the lateral line nerve. Steven (1951) has described cells in the epidermis of the tail of the ammocoete which may act as photoreceptors.

The light response of the ammocoete was chosen for this work because it is a clearly defined response, measurable in terms of the reaction time, occurring in an animal which acts in a limited number of ways and which is probably only sensitive to a small number of stimuli of an elementary nature. The advantage of a light stimulus lies in the accuracy with which its intensity and duration can be measured.

Preliminary experiments on the response to the light stimulus were carried out before proceeding to an investigation of the change produced in the response by the introduction of a thigmotactic stimulus. The thigmotactic stimulus was introduced by allowing the animal to come in contact with sand.
A study was also made by high speed photography of a change in the locomotory movement of the animal when in contact with sand. The development of the response to light and the development of the changed response resulting from the additional thigmotactic stimulus were studied in newly hatched ammocoetes. In the discussion, consideration is given to the events that occur during the reaction time of the light response, and a possible explanation of the modification of the response produced by the thigmotactic stimulus.
II. MATERIAL AND METHODS.

Material.

All experiments excepting those on the development of the light response were carried out on ammocoete larvae of *L. planeri*, about 30-40 mm. in length. Some larger specimens were used in the study of the burrowing movement. The ammocoetes were collected south of Edinburgh, from the river Tyne, Midlothian. They were obtained from the sandy bed of the stream simply by sieving the sand.

A stock of animals was kept in a cool aquarium where there was only a small fluctuation in air temperature. The average water temperature was about 9°C, though this rose 2-3°C during the summer months. The animals were kept in large glass tanks containing a layer of natural river sand about 8 cm. deep covered by an equal depth of water; Edinburgh mains water was used for all purposes throughout this work. A small amount of decaying vegetation was left in the sand, but predatory animals were removed. The animals buried themselves in the sand and required relatively little attention. On the advice of Whiting (private communication) an aeration system was provided for these tanks. He found that given a sufficiently large volume of sand in which to feed and an aeration system which both stirs and aerates the water, the ammocoetes will
actually grow and may attain maturity.

The animals used in a particular experiment were each kept in separate marked crystallising dishes 10 cm. in diameter. The dishes contained water and a layer of sand about 4 cm. deep; they were provided with an aerator. The temperature at which these animals were kept fluctuated about 15°C; though it was higher than that of the stock animals, it approximated to the temperature at which the tests were carried out.

While the animals could be kept alive for long periods under these conditions, presumably feeding on small food particles in the sand, there was little doubt that their condition did deteriorate. The animals became less active, taking longer to bury themselves when disturbed from the sand, and there was a tendency for the reaction time to increase slightly. The animal also succumbed more readily to the adverse conditions of any test. As the deterioration may have been caused by the conditions of the experiment, all animals were returned to the dishes containing sand at the completion of a day's tests and freshly collected animals were used for new series of experiments.

The experiments on the development of the light response were carried out on newly hatched ammocoetes of both *L.* *planeri* and *L.* *fluviatilis* (Linn.). The
material was obtained by rearing artificially fertilized ova. Mature adults of *L. planeri* were collected from local spawning sites. Adults of *L. fluviatilis* were obtained from the river Severn through a commercial dealer at Worcester.

**Apparatus.**

The apparatus provided a light stimulus of variable intensity and duration and was designed for use with three animals. The intensity of the stimulus was controlled by a neutral wedge of graded density, and the duration by a camera shutter. The parts of the apparatus were held in position by clamps attached to a rectangular scaffolding of "Kemiframe." A photograph of the apparatus is shown in Fig.1. A diagram of the arrangement is given in Fig.2, to which reference is made in the following description.

The full radiation of the source *S* was supplied by a 30 watt, 6 volt tungsten filament bulb fitted into a Prior High Intensity lamp (Code No.631). This lamp gave an intense and at the same time evenly illuminated field, and had the further advantages of being a relatively cool source easily operated by an ordinary switch. The lens *L* in the lamp focussed the light from the source in order to pass it through a small area of the wedge *W*, and so prevent variation in the intensity of the stimulating
field which would arise from a broad beam of light passing through the graded wedge. The wedge was a 6 x 1 in. Ilford wedge with an optical density gradient of 0.97/in., bearing an inch scale 0-6 on its upper surface. It was mounted on a blackened brass plate P, in which there was a central aperture slightly smaller in diameter than the breadth of the wedge. The wedge, supported by a runner, was moved across the aperture by means of rubber rollers in contact with its upper surface. The setting of the wedge was read on the scale against a pointer marking the centre of the aperture. Near its focus, the light was also passed through a heat filter $F_1$ of ON 20 Chance glass and a camera shutter C, both being supported by attachments fixed to the mounting plate. Baffles $B_1$, in addition to the brass plate itself, were inserted above and below the plate and on the lamp to prevent light which had not passed through the wedge from reaching the animals being tested.

The expanding cone of light was made parallel by a large convex lens $L_2$. The light was then reflected downwards onto the test position where the animal lay, by a plane mirror $M$, set at an angle of 45 degrees to the optical axis of the apparatus. The mirror could be moved along this axis into three positions $M_1$, $M_2$ and $M_3$, which
enabled three animals to be stimulated successively without moving their containers. The positions of the mirror were marked by stops and were easily found in the dark. Each of the three test positions, I, II and III, were completely screened from each other and from stray light in the room by an inverted black box Sc, supported by the fibre-board base of the apparatus. This box was open on the side of the observer, and admitted the stimulating light to each test position through an aperture in its top. A diaphragm fitted to the aperture, reduced the stimulating field to an area equal to that of the base of the test dish containing the animal; this lessened reflections from the sides of the dish.

The animals were observed in dim light of wavelengths exceeding 650 m\(\mu\) (Ilford filter No.609 Spectrum Deep Red): the ammocoete is relatively insensitive to wavelengths beyond 600 m\(\mu\) (Steven 1950). Only weak illumination was required since the animals were viewed by transmitted light, each appearing as a dark shadow against a deep red background. Each test dish D was supported by a glass plate which admitted light from the observation lamp OL set below, through an area equal in size to the base of the test dish. The lamp housings were small tin boxes perforated to dissipate
the heat produced. Each was covered by an extensive baffle $B_2$ in the centre of which was fixed an Ilford filter No. 609 $F_2$. The light from 6 volt 0.3 amp. bulbs in the lamps, was kept at the minimum necessary to see the animals by a rheostat $R$ fitted into the lamp circuit; the latter was run off the mains supply through a transformer $T$. White filter paper interposed between the bulb and the animal obscured the bright filament; it also served to scatter the stimulating light and so produce a more evenly illuminated field.

Considerable difficulty was encountered during the course of a day's tests in preventing a rise in temperature of the water in the test dishes. Apart from the air temperature in the dark room, in which this apparatus was housed, the rise in temperature was due to heat from the observation lamps and to the enclosed nature of the test positions. ON 20 Chance glass heat filters $F_1$ mounted over the observation lamps and a fan providing a cool air current directed towards the test dishes and the lamps, did however assist in preventing an excessive rise in temperature.

**Calibration of the Apparatus.** The apparatus was calibrated by means of an Eel photometer reading directly in foot candles. Light intensities from 0.5-500 f.c. were measured directly; intensities
below and above this range were calculated from the density gradient of the wedge. The light intensity at each test position was determined as an average of two to three readings for each wedge setting used in any particular experiment. The intensity difference between successive test positions was approximately x 1.5 for the same wedge setting. An intensity determination was made before and after a series of tests, and the intensity values for that series were taken as the mean of the two determinations.

**Use of Apparatus.** The description of the apparatus given above refers particularly to the form providing vertical illumination for three different test positions. The apparatus could be adapted, by removing the mirror, to provide a horizontal beam of stimulating light. The horizontal beam illuminated only a single test position.

**Procedure.**

The light response was investigated by measuring the reaction time at several light intensities, so that when studied under different experimental conditions, both the reaction time at the same intensity and the relation between the reaction time and intensity could be compared. One measurement of the reaction time at each intensity is the minimal requirement for a graph showing the relation
between the reaction time and intensity; it formed the basis on which experiments were planned and is referred to as a set of tests. The information for a particular experiment was obtained by repeating sets of tests on a number of animals.

In a set of tests for one animal the reaction time was measured in random order at four or five intensities, each representing an approximate threefold difference in intensity. A dark adaptation period of at least 60 min. was allowed before the first measurement, and one of 20 min. between each subsequent measurement. This undisturbed period prior to each measurement was also an attempt to eliminate any effect of disturbance in altering the excitability of the animal. The water temperature in the test dishes was measured after the first and last measurements in a set of tests. One or more sets of tests were made in a day on each of several animals selected in rotation from the total number used for an experiment; the same animal was used every two to three days.

In a single test the reaction time was measured by means of a stop watch as the interval from the beginning of stimulation to the first movement of the animal. The stimulus was either of constant duration lasting for 1 or 5 sec., or continued until the animal responded. The camera shutter
controlling the duration of the stimulus was operated by hand: a stimulus of constant duration was timed from the ticking of a clock. Observational illumination was used where the stimulus was of constant duration, but not where it was continuous. Failure to respond within 60 sec. using a constant stimulus, and within 120 sec. using a continuous stimulus, was regarded as a "no reaction."

When the apparatus was used in the form providing vertical illumination the reaction time was measured successively in three animals, one in each test position, at the termination of one dark adaptation period; it was measured at one wedge setting. Owing to the intensity difference between each test position for the same wedge setting, the total number of intensities covered by an experiment was three times the number at which one animal was tested. For horizontal illumination, the number of intensities used in an experiment was the same as the number for one animal, since only one test position was available.
III. THE LIGHT RESPONSE UNDER STANDARD CONDITIONS.

In the preliminary investigation of the light response, the reaction time was measured under standard conditions when the animals were lying freely in a depth of water. The animals were placed in glass crystallizing dishes 5 cm. in diameter containing water 3 cm. deep. They were exposed to a stimulus of 1 sec. duration applied at five intensities in each animal. The apparatus was used in the form providing vertical illumination.

The Reaction Time at Different Light Intensities.

1. Combined results from several animals.

The mean and standard deviation of reaction times obtained at each of five light intensities are shown in Table 1; the data includes results from six animals. The results presented show an increase in the mean length of the reaction time as the light intensity decreased, and also an increase in the variability of the reaction time, expressed by the standard deviation. The reaction time at lower light intensities was both longer and more variable.

The inverse relation between the reaction time and the light intensity is illustrated in the reaction time-intensity graph given in Fig.3, where the reaction time is plotted against the logarithm
of the intensity. Hecht (1918-19a) first pointed out the inverse relation between reaction time and intensity in the light response of Giona, and later found it held for Mya and Pholas (Hecht, 1919-20b and 1927-28). Steven (1950) has previously obtained a reaction time-intensity curve for ammocoetes.

2. Results from individual animals.

The two reaction time-intensity graphs shown in Fig.4, are each constructed from results obtained from a single animal. For each animal, nine reaction times and their mean are plotted at each of five light intensities. Both graphs show the occurrence in the individual animal of the increased length and variability of the reaction time at lower intensities shown by the combined results. When compared, the graphs indicate differences between the two animals, first in the mean length of the reaction time, and secondly in its variability. The reaction time in Animal B was both longer and generally more variable than for the comparable intensity in Animal A (Fig.4). The difference between the mean lengths of the reaction time tended to increase as the light intensity decreased. Animals A and B were selected for presentation since they represent two extremes in individual variation.

The results show a greater variability of the reaction time, both in the same animal and between
different animals, at lower light intensities. The increased variability of the reaction time shown in the combined results was composed of both these sources of variation. The results also show that the reaction time in the one animal was short and consistent, and in the other it was longer and more variable. This suggests that the variability of the reaction time in different individuals depends on the mean length of the reaction time rather than on the absolute light intensity. In its turn, the mean length of the reaction time has been shown to depend not only on the light intensity, but on the animal itself. It is possible that the mean and variability of the reaction time are associated, and that their values depend, at a given intensity, on the excitability of the animal.

The "No Reaction."

A test in which the animal failed to respond was classed as a "no reaction." These tests have been excluded from the results so far presented. The frequency of the "no reaction" in six animals is shown in Table 2; it is given at three ranges of light intensity for tests made at intervals of several days. Throughout the whole period, the frequency of the "no reaction" was greater at the lower intensities. In these six animals out of the total of twelve used for the experiment, the
frequency at the two lowest ranges increased with the number of days after first being tested (Table 2 and Fig.5). The increase is an example of the deterioration in the condition of the animal referred to earlier.

Although the "no reaction" is not without significance, its occurrence reduces the amount of data obtained in an experiment where it is intended to measure the length of the reaction time. This loss can be overcome by using a continuous light stimulus: in 72 tests with an intensity range of 0.92-92.0 f.c. made between 14 and 24 days after the first test, the "no reaction" was absent.

**Spontaneous Movement.**

There was no quality in the movement observed in this experiment to indicate that it was the response to a light stimulus and not attributable to spontaneous movement or mechanical disturbance. The cause of the movement cannot be determined in any single test, but it can be assumed that in the majority of tests the movement was a response to light since the reaction time was correlated with the light intensity. To support this assumption, the probability of one or more spontaneous movements occurring within any 10 sec. interval was calculated from an estimate of the frequency of spontaneous movement; an interval of 10 sec. was chosen since
all reaction times were shorter than this period. The estimate was made by counting the number of separate movements occurring in animals during isolated 2 min. periods. The animals were subjected to the same conditions as the animals tested, and were viewed in dim light which had passed through an Ilford filter No.609. A total of 67 movements were observed in a combined period of 448 min. The probability of the occurrence of one or more spontaneous movements in 10 sec. was found to be 2.5%, assuming the frequency to follow a Poisson distribution. The probability indicated that 1 in 40 of the movements whose reaction time was measured were spontaneous. To minimise mechanical disturbance, the apparatus was reset immediately after the previous test.

Temperature.

Tests were carried out at temperatures ranging from 13.0-21.8°C. In one day, the maximum temperature range was from 14.4-21.0°C, and the minimum from 17.3-17.5°C. The mean daily range in temperature was 3.4°C.

In the light response of Mya, part of the duration of the reaction time varies with temperature (Hecht 1918-19, d). If some part of the reaction time in the ammocoete is also temperature dependent, the fluctuation in temperature in this experiment
could account for the variability of the reaction time. Hecht (1918-19, b) has claimed that the length of the temperature dependent period in Mya is constant over moderate ranges of light intensity: if this were not so in the ammocoete and the period increased at lower intensities where the reaction time was longer, then the fluctuation could also account for the greater variability of the reaction time at these intensities. The present experiment, however, provides no clear evidence to show that the variability of the reaction time can be attributed to the fluctuation in temperature. The results from several animals, presented in Fig.6, show no marked correlation between the length of the reaction time and the temperature at which it was measured for a given light intensity. The tests on the two individual animals (v.s.) were carried out at similar temperature ranges, from 14.0-21.8°C in Animal A, and from 14.4-21.0°C in Animal B.

The temperature range in tests for which the frequency of the "no reaction" is presented are shown in Table 2. They do not suggest that the increase in frequency is related to the temperature at which the tests were made.
IV. MODIFICATION OF THE LIGHT RESPONSE BY A THIGMOTACTIC STIMULUS.

The Delayed Response

The response to light was investigated when a thigmotactic stimulus was applied in addition to the light stimulus. This was carried out by measuring the reaction time in animals which were in contact with sand. Two investigations were made using different methods of establishing contact with sand. In the first, the animal was completely buried in sand contained in a water cell, while in the second it lay on the surface of wet sand. In each investigation, control measurements of the reaction time were made when the animal was lying freely in a depth of water.

Water Cell Tests.

Apparatus. The tests in which the animal was buried were carried out in the container shown in Fig. 7. A glass water cell, 10 x 6 x 1 cm., was fitted with a perspex block and one or more thin glass plates in order to reduce the sectional width of the cell to a size slightly larger than the depth of the animal's body. The remaining space was filled with white sand, which was then covered by a layer of water. An animal introduced into this cell buried itself in the thin section of sand.
It lay, however, with part of its body exposed against the glass face of the cell, so that it remained possible to stimulate the animal with light by illuminating the face of the cell. For the control tests, the animal was placed in the 5 cm. diameter crystallizing dish containing a depth of water.

The positions in which the containers for the animals were placed in the apparatus are shown in Fig. 8. The water cell was mounted in the path of the horizontal beam of light emerging from the convex lens $l_2$. The position of the cell along the horizontal axis of the apparatus was adjusted so that the intensity of light reaching the animal buried in the cell was similar to the intensity at the first test position, for the same wedge setting. The first test position was used for the control tests.

**Calculation of the Light Intensity Reaching the Buried Animal.** The intensity of light passing through the water cell was reduced by the cell and its contents. To obtain an estimate of the intensity of light reaching an animal buried inside the cell, it was supposed that the animal lay at the midpoint of the material responsible for the reduction in intensity; it was assumed that the intensity was reduced by a constant factor per unit
length through this material (Fig. 9 A). The intensity at this midpoint was calculated by reducing the intensity of the light incident on the cell by the square root of the factor by which it was reduced after passing through the whole cell, according to the equation

\[ I_a = I_i \sqrt{\frac{I_t}{I_i}} \]

where \( I_a \) represents the intensity at the midpoint of the material, that is the intensity reaching the animal, \( I_i \) the intensity of the incident light and \( I_t \) the intensity of the light transmitted by the cell.

The method is biased towards an under-estimation of the light intensity reaching the buried animal. Part of the animal's body was exposed to a higher intensity than that calculated, since it lay directly against the front face of the water cell and was not covered by sand. Further, the method assumes the reduction in intensity to be entirely due to the sand, as shown in Fig. 9 A. The reduction was however partly due to the glass plates and perspex block lying behind the sand (Fig. 9 B). The intensity at the midpoint of the sand, where the animal was assumed to lie, was therefore higher than the method allows (Fig. 9 A and B). The under-estimation opposes the change in the reaction time observed in this experiment.
Procedure. The reaction time was measured at four light intensities in the buried tests and at four similar intensities in the control tests; a continuous light stimulus was used in both types of test. For one animal, a control test was made at two intensities before it was introduced into the water cell; here, single tests were carried out at each intensity. The animal was returned to a depth of water where a control test was made at each intensity. The animal remained in the water cell for approximately two hours.

Spontaneous Movement. Where reaction times are long and variable, it is possible that some may represent a spontaneous movement rather than a response to light. A reaction time was regarded as a response to light if it lay within the time interval during which there was a 5% or lesser probability of the occurrence of one spontaneous movement. A reaction time longer than this interval was regarded as representing a spontaneous movement. The interval was calculated as 5% of the average period $p$ during which one spontaneous movement occurred. $p$ was derived from the observed frequency of spontaneous movement according to the equation

$$p = \frac{m}{t}$$

where $m$ is the number of spontaneous movements observed in time $t$. 
The observations of the frequency of spontaneous movement for animals buried in the water cell were carried out in conjunction with the tests, described later, which required the use of observational illumination. The water cell was illuminated by dim light which had passed through an Ilford filter No.609. As the illumination was insufficient to enable an animal lying within the sand to be distinguished, the observations were made on occasions when the tip of the head protruded slightly above the level of the sand. The animals were watched for isolated 3 min. periods during which the number of separate movements were counted. A total of 28 movements were observed in a total period of 180 min. The maximum length of the reaction time regarded as a response to light, calculated as above, was \( \frac{180}{28} \times \frac{60}{20} = 19.3 \) sec. (Assuming the frequency of spontaneous movement to follow a Poisson distribution, the probability of the occurrence of one or more spontaneous movements in 19.3 sec. was 4.9%). Reaction times longer than 19.3 sec. have been classed as "no reactions" in the results presented below. The correction affected 43% of the results.

Results.

The individual reaction times obtained in the buried tests, and the total number of "no reactions"
are shown in Table 3. They are shown at four light intensity classes; each class combines the results obtained at three similar intensities. The mean and standard deviation of reaction times obtained in the control tests are shown in Table 4; the "no reaction" was absent. They are shown at four intensity classes which each combine the results obtained at two similar intensities.

The reaction times obtained in the buried tests were very much more variable and generally longer than those obtained in the control tests. The mean reaction time was, on average, approximately four times that of the control tests. The "no reaction" occurred in 57% of the total number of buried tests, but not at all in the control tests.

The difference between the results of buried tests and control tests carried out at similar intensities may be stated by the proportion of buried tests whose results differed from those of the control tests. A result was regarded as different either when the "no reaction" occurred, or when the reaction time was outside the limits of 95% of the control reaction times. The limits are set by a function of the standard deviation away from the mean. The function varies slightly according to the number of items from which the standard deviation is calculated, but was here
approximately two standard deviations. Reaction times lying within these limits are referred to in the text as "short" reaction times, and those lying outside are referred to as "long" reaction times. Table 5 shows the number of "short" and the number of "long" reaction times obtained in the buried tests, and also the number of "no reactions." They are shown at four light intensities, together with the upper limit of the "short" reaction time calculated from the control reaction times obtained at four similar intensities. The number of "long" reaction times and "no reactions" combined is shown as a percentage of the total results in the final column of the table.

The results obtained in the buried tests were distributed into a small proportion which were similar to those of the control tests, and a large proportion which differed. Where the results were similar, the reaction times were short; where the results differed, the reaction times were either long and variable or were not obtained at all. The distribution is regarded as characteristic of what is referred to as the delayed response.

Wet Sand Tests.

The second method of establishing contact with sand derives from the type of movement observed in animals placed on the surface of wet
sand. This movement, referred to as the burrowing movement and described in a later section, was observed in the water cell when the animal burrowed through the sand. Its occurrence is regarded as evidence that contact with sand has been established.

The animal was placed on a thin layer of sand contained in the 5 cm. diameter crystallizing dish. It was covered by a shallow layer of water, insufficient to allow it to swim. The animal lay in a depression in the sand with the water film just touching its dorsal surface. The control tests were carried out in a depth of water only. The apparatus was used in the form providing vertical illumination.

The sand tests and control tests were carried out at four light intensities in each animal using a continuous light stimulus. A set of single control tests at each intensity was made before and after a set of single sand tests at each intensity in the one animal. If any of the reaction times obtained in the control tests following the sand were unusually long, the results of those sand tests were rejected on the basis that the animal was affected by the adverse conditions of the experiment. The results of 12 sand tests were rejected for this reason out of a total of 96 tests that were carried out. Six animals were used for the experiment.
Spontaneous Movement. The observations of the frequency of spontaneous movement for animals lying on wet sand were carried out in dim light which had passed through an Ilford filter No. 609. The animals were watched for isolated 5 min. periods during which the number of separate movements were recorded. A total of 28 movements were observed in a combined period of 200 min. The distinction between a reaction time representing a spontaneous movement and one representing a response to light was calculated from this data in the manner described for the water cell tests: it was 21.4 sec. (Assuming a Poisson distribution, the probability of the occurrence of one or more spontaneous movements in 21.4 sec. was 4.9%). Reaction times longer than 21.4 sec. have been classed as "no reactions" in the following results of which 43% were affected by the correction.

Results.

The results obtained in the sand tests have been classed, as described in the previous experiment, into "short" reaction times, "long" reaction times and "no reactions." The numbers lying within each class are shown in Table 6 at eight light intensities. The results obtained in tests carried out at similar intensities have been combined. The number of "long" reaction times and
"no reactions" is presented in addition as a percentage of the total number of results. The table also shows the upper limit of the "short" reaction time and the mean and standard deviation of the control reaction times from which it was calculated, for the same eight light intensities. The "no reaction" was absent in the control tests.

The results obtained in the sand tests were composed of a small proportion of "short" reaction times and a large proportion of "long" reaction times and "no reactions." Although not individually presented, the reaction times included in the "long" reaction time class were extremely variable. The proportion of "long" reaction times and "no reactions" represents the proportion of results which differed from those of the control tests.

These results were essentially similar to those obtained in the buried tests in the previous experiment. It is likely that the occurrence of the delayed response depends on the presence of a contact stimulus, since it occurred where independent methods of establishing contact with sand were employed.

Wet Perspex Tests.

A delayed response was obtained in the previous experiment when close contact with sand was established by placing the animal in a shallow...
layer of water overlying a sand substratum. The experimental conditions, apart from the contact with sand, may have contributed towards the delayed response. This was investigated in the present experiment where the experimental conditions, excepting the layer of sand, were reproduced. The reaction time was measured in animals placed in a shallow layer of water overlying a smooth perspex substratum.

The tests were carried out in flat-bottomed dishes, 5 cm, in diameter, prepared by turning a depression out of sheet perspex. The amount of water added to the dish was such that the surface of the water film just touched the dorsal side of the animal. Control tests were carried out in a depth of water. The apparatus was used in the form providing vertical illumination.

The reaction time was measured at the same three light intensities in both the tests carried out on the wet perspex and the control tests. A continuous light stimulus was used. Control tests were made before and after the tests on the wet perspex. Six animals were used for the experiment.

Spontaneous Movement. Observations of the frequency of spontaneous movement were carried out for animals lying in a shallow layer of water on a base of smooth perspex. The animals were observed
in dim red light (Ilford filter No.609) during isolated 5 min. periods. A total of 61 movements occurred in a combined period of 205 min. The maximum length of the reaction time regarded as a response to light was 10.1 sec. (The probability of the occurrence of one or more spontaneous movements in 10.1 sec., assuming a Poisson distribution, was 4.8%). 7% of the reaction times obtained in this experiment were longer than 10.1 sec.; they have been classed as "no reactions" in the following results.

Results.

The numbers of "short" reaction times, "long" reaction times and "no reactions" obtained in the perspex tests are shown for three light intensities in Table 7. The table also shows the upper limit of the "short" reaction time, and the mean and standard deviation of the control reaction times obtained at the same intensities.

The proportion of results which differed from those of the control tests was smaller in this experiment than in the wet sand experiment. In this experiment, 61% of this proportion was composed of reaction times less than 5 sec. compared with 8% in the wet sand experiment. It seems likely that the fact of an animal lying in a dish containing a shallow layer of water contributes little towards
the delayed response. Its occurrence must be attributed mainly to the presence of sand. It is possible that the longer reaction times in the perspex tests were due to the contact established with the base of the dish.

The Delayed Response in Partially Buried Animals.

An investigation was made to determine whether the delayed response was associated with the application of a thigmotactic stimulus to a particular region of the body. The investigation was confined to the anterior and posterior parts of the body and was carried out by measuring the reaction time, first when the head and branchial region of the animal were buried in sand, and secondly when the tail was buried.

Head Buried Tests.

The tests were carried out in the dish illustrated in Fig.10. A circular block of perspex was fitted inside a 5 cm. diameter crystallizing dish, leaving a space around the perimeter of the dish to be filled with white sand. An animal introduced into this dish buried only the anterior part of its body; it was prevented from burying itself completely by transverse partitions
in the sand, arising as spokes from the central block. The water level in the dish, though always well above the sand, was adjusted so that the exposed part of the body did not extend vertically but lay approximately at right angles to the beam of incident light. The area of the body in contact with sand varied in different tests, but always included the head and branchial region and some part of the body posterior to this region. Control tests were carried out in a depth of water. The apparatus was used in the form designed for vertical illumination.

The reaction time in the head buried tests and control tests was measured at five light intensities in each animal using a stimulus of 1 sec. Head buried tests and control tests were carried out on alternate days in a total of three animals.

**Spontaneous Movement.** Observations of the frequency of spontaneous movement were carried out for animals in which the head was buried in sand. The animals were illuminated by dim red light (Ilford filter No.609) and were watched during isolated 5 min. periods. A total of 42 movements were observed in a combined period of 220 min. All the reaction times obtained in tests carried out under these conditions were less than 5 sec.: the probability of the occurrence of one or more
spontaneous movements in any 5 sec. period, calculated from the above data assuming a Poisson distribution, was 1.6%.

Results.

The numbers of "short" and "long" reaction times obtained in the head buried tests are shown at nine light intensities in Table 8. The mean and standard deviation of the control reaction times and the upper limit of the "short" reaction time are also shown for the same light intensities. The reaction times obtained in the head buried tests were almost exclusively "short." The few reaction times included in the "long" reaction time class were all less than 5 sec.

The "no reaction" occurred in both the head buried tests and the control tests. The total number of "no reactions" obtained in both types of test are shown in Table 9. The value of Chi-Square calculated from these results showed that if the discrepancy between the proportion of "no reactions" in each type of test were not due to the different experimental conditions, such a discrepancy or greater could be expected in 2-5% of similar experiments. Although the discrepancy is therefore probably significant, the actual difference between the proportion of "no reactions" was small. The "no reaction" occurred in only 12% more head buried
tests than control tests.

The results obtained in the head buried tests did not differ markedly from those of the control tests. This indicates the absence of the delayed response. The reaction times that were obtained in the head buried tests were mostly "short;" the "short" reaction times formed 41% of the total results, compared with 18% in the water cell tests and 10% in the wet sand tests where the delayed response occurred. The occurrence of the "no reaction" in the head buried tests was not an indication of the delayed response, since the proportion of "no reactions" was only slightly greater than that obtained in the control tests. Its occurrence in the control tests was due to the use of the 1 sec. stimulus; in the head buried tests it may be largely attributed to the same cause.

Tail Buried Tests.

The tests in which the posterior part of the body was in contact with sand were carried out in conjunction with the water cell tests described earlier. At times, the anterior part of the body protruded above the level of the sand in the water cell, leaving only the posterior part in contact with sand. The results of tests carried out on these occasions are reported here; the part of the body remaining in the sand did not include the
post-branchial region. The light intensity reaching the partially buried animal was regarded as the same as that reaching the completely buried animal since the tail, the region most sensitive to light (Young 1935), remained buried in the sand.

Results.

The numbers of "short" reaction times, "long" reaction times and "no reactions" obtained in the tail buried tests are shown in Table 10 at four light intensities. Details of the control tests and the upper limit of the "short" reaction time are given earlier in Tables 4 and 5.

The results of the tail buried tests were distributed into a small proportion of "short" reaction times, similar to the control tests, and a large proportion of "long" reaction times and "no reactions," which differed from the control tests. The reaction times included in the "long" reaction time class were highly variable. These results were similar to those obtained in the water cell and wet sand tests. They indicate the occurrence of the delayed response.

The experiments on partially buried animals show that the delayed response is associated with the application of a thigmotactic stimulus to a particular region of the body. The region is located in all or part of the posterior half of the body.
The Relation between the Delayed Response and the Intensities of the Light and Thigmotactic Stimuli.

The response to light measured under standard conditions has been shown to be related to the intensity of the light stimulus. It is possible that the response obtained when a thigmotactic stimulus is applied in addition to the light stimulus also shows a relation to the intensity of the light stimulus, and further, a relation to the intensity of the contact stimulus. If this were so, given magnitudes of the response could be related to the intensities of the two stimuli. The necessary data could be obtained by measuring the magnitude of the response either at varying intensities of contact stimulus for several constant values of light intensity, or conversely, at varying intensities of light stimulus for several constant values of contact intensity (Fig. 11). The change from the response obtained under standard conditions to the delayed response obtained where contact with sand is established, described previously, would then become the special case of the change in the response where the intensity of the contact stimulus increases from zero to some fixed value. It is therefore convenient to regard the response obtained under each experimental condition, not as two different types of response, but as a variation in
the same response obtained at different values of intensity of the contact stimulus; the response obtained under standard conditions becomes the response obtained where contact is zero.

The magnitude of the response can be measured in terms of the reaction time. It was therefore necessary to select a suitable standard by which a change in the reaction time could be measured in these experiments; in order to relate the response to the intensities of each stimulus. Where contact with sand was established, long and variable reaction times and "no reactions" were obtained. The change in the reaction time could not then be measured by the mean length of the reaction time, as under standard conditions. It could however be measured by fitting the results into a frequency distribution. The distribution chosen contained two classes. The first class included reaction times of 19.3 sec. and less, and the second, reaction times above 19.3 sec. and the "no reaction;" the "no reaction" was assumed to be infinitely long reaction time. In the presentation of the results, the change in the reaction time is represented by the proportion of results falling within the first class, since an increase in this proportion corresponds to an increase in the response to light.

The use of a frequency distribution in revealing a
relation to the light intensity has already been demonstrated for the results obtained under standard conditions, where the proportion of "no reactions" decreased as the light intensity increased.

The same distinction between the classes was applied to the results obtained under different experimental conditions, so that a strict comparison between the results could be made. For the same reason, reaction times representing a spontaneous movement were excluded from the first class. If they were not excluded, the number of results lying within this class would be altered by a varying amount depending on the frequency of spontaneous movement occurring under different experimental conditions. The distinction between the classes could not therefore be made higher than the lowest reaction time regarded as a spontaneous movement for all the experimental conditions employed. The lowest reaction time was that calculated for animals buried in the water cell; it was 19.3 sec. For tests carried out in the water cell, the distinction between the classes corresponds to the distinction between a reaction time and a "no reaction." The first class is therefore conveniently referred to as the proportion of "reactions."
Varying the Intensity of the Contact Stimulus.

An experiment is described in which the reaction time was measured at three intensities of contact stimulus at a single constant light intensity. An attempt was made to vary the intensity of the contact stimulus by using different sands each composed of particles of a standard size.

White sand was separated by means of standard geological sieves. Three sands, whose average particle sizes were 425µ, 214µ and 100µ, were selected for the experiment. Tests were carried out in the manner described for the wet sand experiment where contact with sand was established by placing the animals on a thin layer of sand covered by a shallow layer of water. Control tests were carried out in a depth of water before and after the sand tests in each animal. Unusually long reaction times occurred in the control tests following 15 out of a total of 95 sand tests; the results of these 15 tests were rejected. Eight animals were used for the experiment, each being tested with each of the three sands.

Owing to the intensity difference between each test position, the experiment was carried out at three light intensities ranging over 0.4 log. units. The intensity range was however regarded as a single intensity in the assessment of the results, since
it represented 1/7 of the intensity range in the experiment where the light intensity was varied, being less than the intensity difference between successive light intensities.

Results.

The results are presented in Table II. The table shows the percentage of "reactions" obtained in tests where three types of sand were used, and the percentage obtained in control tests where sand was absent.

The results show that the proportion of "reactions" decreased where the intensity of the contact stimulus was raised above zero. They also show that the decrease continued as the size of the sand particle increased. The results would indicate a continuous decrease in the response as the intensity of the contact stimulus increases, if the larger sand particle represents an increased intensity of contact stimulus. Such a postulate might be expected to follow from the increased pressure at fewer contact points resulting from the extra loading on each sand particle.

The trend observed in this experiment requires further investigation, with a view to employing the size of the sand particle as a means of investigating the relation between the response and the intensity of the contact stimulus at constant light intensities.
The effect of the size of the sand particle on the intensity of the contact stimulus could however be regarded equivocally. It therefore remained to measure the response at varying light intensities where the intensity of the contact stimulus was zero, and at some unknown intensity represented by contact with sand. This was carried out in the following experiments.

Varying the Intensity of the Light Stimulus.

The response and its relation to the light intensity is compared in the presence and absence of a contact stimulus. Curves relating the proportion of "reactions" and the light intensity were obtained from tests in which a continuous light stimulus was used.

(a) Contact Stimulus Absent.

The reaction time was measured at light intensities lower than those previously used. At these intensities the reaction time was long, its variability increased and the "no reaction" occurred. The proportion of "reactions" was no longer maximal so that a change in the reaction time could be assessed by this standard.

Tests were carried out in a depth of water using the apparatus in the form providing vertical illumination. The reaction time was measured at five light intensities in six animals.
Spontaneous Movement. The observations of the frequency of spontaneous movement for animals lying in a depth of water have been described in connection with the tests carried out under standard conditions. The highest reaction time regarded as a response to light calculated from this data was 20.1 sec. The calculation was required in order that the choice of the distinction defining a "reaction" could be made to exclude reaction times which represented a spontaneous movement.

Results.

The percentage of "reactions" obtained in tests carried out at five light intensities are shown in Table 12. The results show an increase in the proportion of "reactions" as the light intensity increased. The results are presented graphically in Fig.12, curve Co.

(b) Contact Stimulus Present.

An experiment in which the reaction time was measured at several light intensities in the presence of a contact stimulus has already been described in the water cell tests. The intensity range of the experiment was here extended by increasing the intensity of the light stimulus.

The full radiation of the source was provided by a 500 watt tungsten filament bulb. Slight readjustment was made to the lens system of the
apparatus in order to focus the beam from the source on to a small area of the wedge, and to direct the cone of light emerging from the camera shutter on to the water cell. The reaction time was measured at two light intensities which were higher than those previously used. The experiment was carried out on five animals. In other respects, the experiment was performed in the manner described for the water cell tests.

Results.

The percentage of "reactions" obtained in the present experiment and in the earlier water cell experiment are shown in Table 13. They are shown at six light intensities. The proportion of "reactions" increased as the light intensity increased. The relation is plotted in Fig. 12, curve C₁.

The Light Response in the Presence and Absence of a Thigmotactic Stimulus.

The curves presented in Fig. 12 show the relation between the proportion of "reactions" and the logarithm of the light intensity in the presence and absence of a contact stimulus. They show that a higher light intensity was required to produce a given proportion of "reactions" in the presence of contact than in its absence. They also show that a given change in the proportion of "reactions"
where contact was present required approximately three times the logarithmic increment in light intensity required to produce the same change where contact was absent. Further, the relation between the proportion of "reactions" and intensity was not linear either in the presence or absence of contact.

The effect of contact on the light response is such that the same response can be obtained in the absence of contact by lowering the light intensity. A single measure of the effect of contact cannot however be obtained from the decrement in light intensity required to produce the same response in the absence of contact, since contact also alters the relation between the response and intensity: the decrement varies at different levels of the response. The different relation between the response and intensity in the presence and absence of contact can be explained in two ways. The contact stimulus may reduce the effectiveness of any given logarithmic increment in light intensity in producing a change in the proportion of "reactions." Alternatively, since neither of the relations is linear, it is possible that the different relation in the presence of contact is due to the higher intensity range over which the proportion of "reactions" was measured. The latter alternative implies an established relation between the response
and intensity, and that the effect of contact is simply to reduce the absolute value of the response.

The Proportion of "Reactions" using Stimuli of Constant Duration.

The tests in which the reaction time was measured in the presence of a contact stimulus were generally carried out using a continuous light stimulus. How far all the energy of the continuous stimulus is necessary to produce a given proportion of "reactions" was investigated in the present experiment. The proportion of "reactions" was assessed from tests in which stimuli of 1 sec. and 5 sec. duration were applied at a single high intensity.

Tests were carried out on animals buried in the water cell. The source was the 500 watt tungsten filament bulb. The observational illumination required to see the animal after the stimulus had been applied was provided by light which had passed through an Ilford filter No.609. As the body of the animal could not be distinguished in the sand, the tests were made when the tip of the head protruded slightly above the level of the sand. The experiment was carried out on five animals.

Results.

The percentage of "reactions" obtained in
tests using stimuli of 1 sec. and 5 sec. duration are presented in Table 14. The percentage of "reactions" obtained at a similar intensity using continuous stimuli is also shown.

Stimuli of 1 sec. duration produced approximately one quarter of the proportion of "reactions" obtained where the stimuli were continuous; stimuli of 5 sec. produced more than half of this proportion. The results suggest that the quantity of light energy falling on the animal in the first second of the continuous stimuli accounts for a quarter of the total number of "reactions" obtained. The energy of the following four seconds accounts for nearly a further third of the total number of "reactions." At least some of the energy reaching the animal after the first five seconds of the continuous stimuli is required to produce the remainder of the "reactions."

The light energy reaching the animal in the first few seconds of the stimulus appears to be more effective in producing a response than that reaching the animal later. This interpretation corresponds with the known adaptation of the nerve impulse discharge in photoreceptors (Hartline, 1941).
V. THE BURYING MOVEMENTS OF THE AMMOCOETE.

The water cell method of applying a contact stimulus depends on the natural ability of the ammocoete larva to bury itself in sand. The movements by which this burial is achieved and the character of the movement occurring beneath the sand are described in the present section. The description is based on cine-photographs of animals burying in the water cell, and of animals moving over the surface of wet sand. The thin section of sand in the water cell made it possible to follow the progress of the animal through the sand. Photographs were also taken of the swimming movement.

Initial Penetration.

Before the burying movements begin, the animal swims about in the water layer with its head inclined downwards towards the underlying sand. The swimming movement of the ammocoete (Fig.13) closely resembles the swimming movement of the eel described by Grey (1933). Contractions pass posteriorly along the muscles on alternate sides of the body, so that at any instant it lies in a sinusoidal curve. The crest of any curve recedes relative to the ground as the animal moves forward. Each part of the body including the head is moving transversely to the axis of progression.
The initial penetration of the sand (Fig. 14 A-G and 15 A-H) is achieved by a fast swimming movement in which the head is applied to a particular point in the sand; the contraction waves pass rapidly down the body. The transverse movement of the head is reduced, yet it remains sufficient to displace the sand. This displacement of the sand and the thrust developed by the swimming movement enable the anterior part of the body to penetrate the sand. The movement lasts for approximately 1 sec., ending in small animals (c. 3 cm. length) when a third of the body is buried; in larger animals, the movement stops when a smaller proportion of the body is buried.

The initial penetration movement has been observed in the absence of sand. Animals swimming in a dish containing water only direct the head almost vertically downwards and execute a fast swimming movement of short duration. This frequently occurs at the junction between the base and sides of the dish.

**Subsequent Penetration.**

After the fast swimming movement, the buried part of the body begins a burrowing movement whereby a sinuous path is traced out beneath the sand. Initially (Fig. 14 H-P and 15 I-K), this is a
lateral movement of the head associated with a shovelling action of the lips (cf. Fig. 18); it is accompanied by a slow lashing of the exposed tail. When this movement has enabled a sufficient length of body to penetrate the sand, the buried part of the body develops a curve to one side (Fig. 14 Q-R and 15 L-N). This single curve is then extended into an S-shape as the body moves forward and a curve towards the opposite side is added. The early curves may be obliterated as new curves involving the contraction of more myotomes develop when more of the body enters the sand (Fig. 15 O-T). The curves may, on the other hand, be perpetuated and the original sinusoidal track merely extended anteriorly as the animal buries itself further (Fig. 14 S-V). A completely buried animal can occupy a sinuous curve extending over three wavelengths.

Serial contractions continuing in the exposed part of the body can produce a lashing movement of the tail (Fig. 15 P-T); these contractions are gradually arrested as more of the body is buried. Finally, the exposed body is drawn passively beneath the sand, retaining the form of the arrested contraction until it does so. The exposed tail commonly lies along the surface of the sand before it is buried, the tail tip pointing in the direction in which the head is progressing (Fig. 14 R-T).
These later penetration movements do not always occur continuously. The animal may take up to 1 min. to bury itself completely if the burying movements are interrupted by several periods of inactivity. When the movements are continuous, complete burial can occur within 2 sec.

The Burrowing Movement.

The burrowing movement occurring beneath the sand in the water cell is more conveniently studied in animals moving over the surface of wet sand. Photographs of animals moving over this medium (Fig. 16) show that serial contractions pass down the muscles on alternate sides of the body, throwing it into a sinuous curve; the crest of each curve remains stationary relative to the ground. This is confirmed by the single line track left by an animal moving over wet sand (Fig. 17); it shows that each part of the body follows the path traced out by the region anterior to it. This is not however true for the head. It executes a lateral movement of small amplitude across the track followed by the rest of the body. This movement of the head is accompanied by a movement of the oral lips. When viewed from above, the original rectangular shape of the snout appears transformed into a point directed towards the side to which the head is moving. As the head swings to the opposite side,
the lips reverse their position. The movements of the head and lips are illustrated in Fig. 18. They are the movements that occur during the burying of the ammocoete soon after a short length of body has penetrated the sand.

The sinuous form of the ammocoete moving through or over sand corresponds to the bodily form theoretically required for the progression of the grass snake over an irregular surface. Gray (1946) has shown that given a sinuous bodily form the progression of the snake depends on the presence of external resistances against which the tension set up in the axial musculature can act; the external resistances are supplied by the uneven surface. It can be assumed for the ammocoete that the sand acts as a resistance opposing the tension of the axial musculature, and that the propulsive force for progression is derived from a "pattern of muscular effort" fundamentally similar to that occurring in the grass snake (Gray and Lissmann, 1950). The progression of the ammocoete, however, though mainly dependent on the propulsive force developed in the body muscles, may be assisted by the lateral head movement and lip movement in displacing the sand.

The main features of the burrowing movement can be seen both in animals in the water cell and in
animals moving over wet sand. There are, however, differences in the number of curves exhibited by the body under each condition. Measurements obtained from animals moving over wet sand show that the wave-length of the sinuous track varies directly with the length of the animal: different animals exhibit the same number of curves, each animal occupying approximately $\frac{1}{2}$ wavelengths under these same conditions (Fig. 19A). In the water cell, the animal may occupy up to 3 wavelengths (Fig. 19B). The greater number of curves occurring in animals in the water cell might be the result of a greater resistance to progression. The burying of the ammocoete under natural conditions probably only differs from the preceding description of burying in the water cell in respect of such differences relating to precise bodily form, and also in the tendency of the animal to burrow horizontally and never deeper than 2 cm.

The burying of the ammocoete effects a change in the position of the animal from a fluid to a more compact medium. The external resistances preventing progression differ in each medium, so that the muscular effort required for progression must also differ. The muscular effort of the swimming movement is fitted to overcome the resistance of water, and that of the burrowing movement
to overcome the resistance of sand. Each movement will therefore be unable to effect progression in the inappropriate medium. Certain differences between the swimming and burrowing movements can be observed. Serial contractions pass more slowly down the body in the burrowing movement, and the crests of the sinuous curves do not recede relative to the ground as in the swimming movement. The amplitude of the sinuous curves is larger in the burrowing movement, compared with the amplitude in a straight forward swimming movement. The burrowing movement features a lateral head movement and lip movement which have not been observed in the swimming movement. Since the swimming movement cannot effect progression through sand, the burying of the ammocoete depends on a sufficient length of the body being transferred to the sand for the muscular effort of the burrowing movement, in the buried part of the body, to overcome the resistance of the sand. The burying movements of the ammocoete can be regarded as satisfying this requirement. The initial fast swimming movement enables a certain length of body to penetrate the sand. The lateral head movement and lip movement then facilitate further penetration, until the length of body in contact with sand is sufficient to permit the formation of at least one curve showing an increase
in curvature towards the head (Fig. 14 R and 15 M); such a curve is the minimum requirement for forward progression in the similar serpentine movement of the grass snake (Gray, 1946).

The Movement Occurring in Response to Light.

The type of movement occurring in response to light depends on the experimental conditions and is the same as the locomotory movement for those conditions. The movement of the response is always a swimming movement where the animal is in a depth of water, and always a burrowing movement where the animal is in contact with sand. In the water cell or on the surface of wet sand, the movement was usually of short duration, the animal moving only a few millimetres. The movement was often in a backward direction.

It has been shown that the reaction time of the response also depends on the experimental conditions; it is short where the animal is in a depth of water and longer or absent where the animal is in contact with sand. In tests, this leads to the occurrence of the short reaction time with the swimming movement and the longer reaction time with the burrowing movement. It is probable, however, that the length of the reaction time, with which the present work is concerned, is not dependent on the type of movement since a short reaction time
can occur in association with a burrowing movement. The association has been observed in the water cell and wet sand tests; although a burrowing movement always occurred in these tests, some short reaction times were obtained. The wet perspex tests provided further evidence. Here, the small amount of water at times prevented the animal from swimming and the burrowing movement then occurred. The burrowing movement was distinguished chiefly by the slow coiling of the body and the lateral movement of the head. The swimming movement was evident from the rapid serial contractions and the skidding of the body over the surface of the perspex, which gave the animal the appearance of floundering. It was possible to distinguish a burrowing movement in 24 of the perspex tests; the results of these tests were composed of 12 "short" and 8 "long" reaction times, and showed that a short reaction time can occur with a burrowing movement. Further evidence of the burrowing movement occurring with a short reaction time was provided by the head buried tests. The movement of the response was a slow deflection of the exposed tail. This movement occurs in the later stages of burying and is associated with a burrowing movement beneath the sand. The reaction time in these tests was mostly short.

The swimming movement, on the other hand, only
occurs when the reaction time is short. This might be the result of the swimming movement and the short reaction time being inseparable experimentally. They would be inseparable if the change from the swimming movement to the burrowing movement occurred where the body could be engaged with relatively few contact points, as seems probable, and if the change from the short to the long reaction time occurred when contact was more extensive. The invariable occurrence of a short reaction time when the response is a swimming movement to which this would lead is explained in Fig. 20. The proposed reason for regarding the swimming movement and short reaction time as inseparable would imply that contact, acting as a resistance, produces a burrowing movement through the activity of the muscle proprioceptors, and that the longer reaction time is a true thigmotactic effect of a surface applied to the body.
VI. THE DEVELOPMENT OF THE RESPONSE TO LIGHT.

Observations of the behaviour of newly hatched ammocoetes of _L.fluviatilis_ suggested that their response to light is initially weak or absent. When these animals were first exposed to light, it was noticed that movements were slow to appear and occurred in only a few animals. Later, light produced immediate intense activity in all animals. The present experiments were made in order to describe this apparent increase in sensitivity as a change in the reaction time. The change was measured over a particular range of light intensity during a period beginning shortly before hatching.

Control observations of the frequency of spontaneous movement and the development of movement were made to determine, respectively, how far the reaction times obtained represented a spontaneous movement rather than a response to light, and how far they depended on the ability to move. Since measurements of the reaction time were repeated on the same animals, the effect of previous exposure to light on the appearance of the fully developed response was tested by using animals which had been previously kept in darkness. In addition, a short account is given of the morphological development of the ammocoete over the relevant period.
An investigation was also made to show whether the delayed response, which occurs in older ammocoetes when both a light and thigmotactic are applied, develops in association with the light response.

The majority of the experiments were carried out on *L. planeri* in May and June 1955; preliminary experiments using *L. fluviatilis* took place in the same months in 1954. The experiments differed in only minor points of procedure and are described together; a distinction is however made between the two species in the presentation of the results.

**Materials.** The ammocoetes were obtained by rearing artificially ova. The embryos were placed in large open aquarium tanks with either a supply of running water or an aeration system. The majority were kept in indirect sunlight and were exposed to the natural diurnal variation in illumination. A small batch of *L. planeri* were kept in darkness in an enclosed tank.

The temperature at which the embryos were reared varied between 9°C and 16°C. The early development of all embryos took place within the lower part of this range. The later development of some embryos was hastened by bringing them to a higher temperature, in order to provide material for successive investigations from ova of the same fertilization.
Naturally hatched ammocoetes were used for the experiments on *L. fluviatilis*; they were hatched artificially for those on *L. planeri*. This was done without damage to the animal, simply by tearing the egg capsule with fine forceps.

**Procedure.** The development of the response was studied in individual animals by a series of repeated observations; the animals lay freely in a depth of water. The observations were begun three to four days before hatching in six specimens of *L. planeri*, and shortly after hatching in twelve specimens of *L. fluviatilis*. The observations of *L. planeri* were repeated every 12 hours, and those of *L. fluviatilis* at least once every 24 hours until the reaction time became identical with that obtained in older ammocoetes. The observations involved the following procedure.

1. The reaction time was measured at four light intensities in each specimen of *L. planeri*, and five intensities in each one of *L. fluviatilis*. A dark adaptation period of exactly 60 min. was allowed before the first measurement, and one of 30 min. between each subsequent measurement. A series of such measurements constituted a single set of tests. A continuous light stimulus was used; the maximum duration was 120 sec.

2. The frequency of spontaneous movement was
measured by counting the number of separate movements made by animals during isolated 3 min. periods. The animals were subjected to the same conditions as the test animals, except that they were illuminated by dim light which had passed through an Ilford filter No.609. The observations were made before each single measurement of the reaction time.

3. The length of the animals was measured at the completion of one set of tests. Measurements were made under a microscope using a calibrated grid eyepiece.

The apparatus was used in the form designed to provide vertical illumination.

**Spontaneous Movement.** The data from the repeated observations were combined to provide estimates of the frequency of spontaneous movement for three separate periods during the development of the response. From each of these estimates, the distinction between reaction times representing a spontaneous movement and those representing a response to light was calculated in the manner described for the water cell tests. Each distinction was then applied to the reaction times obtained during the respective period over which the frequency was estimated. Reaction times representing a spontaneous movement have been excluded from the results, the tests where they occurred being regarded as void.
The estimates were made separately for three periods in order to eliminate any change in the reaction time that arose from a change in the frequency of spontaneous movement. Each estimate covered a period in which the reaction times were similar but differed from those of the other two periods. In the first period reaction times were rarely obtained, but were long where they did occur. In the second period reaction times were frequently obtained, though they remained long. Reaction times were always obtained in the third period where they were short.

The maximum length of the reaction time regarded as a response to light and the frequency of spontaneous movement from which it was calculated are shown for each of the three periods in Table 15; the percentage of reaction times rejected after the distinction had been applied is also shown. The extent of each period is indicated by the length of the animal. The information is given separately for the two species. Although there was an increase in the frequency of spontaneous movement, it did not lead to a high percentage of rejected reaction times, since the majority of the reaction times decreased in advance of the increase in spontaneous activity.

**Length.** The development of the response to
light has been related to the length of the animal. Length was chosen in preference to time from hatching or time from fertilization, because it enabled the results obtained from animals reared at different temperatures to be combined, and obviated the difficulty arising in those animals which were hatched artificially. Fig. 21 shows that the reaction times of animals from two populations reared at different temperatures, approximate more closely when plotted in relation to length than when plotted in relation to time from hatching. Time from fertilization was known to be unsuitable for this combination, since material from the same fertilization was used for successive investigations. Measurements of the Reaction Time.

The results of the measurements of the reaction time are presented in Table 16 for L. planeri, and in Table 17 for L. fluviatilis. The tables combine the results from six animals of L. planeri, and twelve of L. fluviatilis; in eight of the latter, however, the measurements did not cover the period when the first response appeared. The tables show single measurements of the reaction time and the number of "no reactions" for several different light intensities, for a series of empirically chosen length classes which do not obscure the development of the response. The initially large length classes
cover periods in which there was no change in the response.

The results show a change in the reaction time and a change in the frequency of the "no reaction" as the animal developed.

1. The Change in the Reaction Time.

The change in reaction time obtained for *L. planeri* and *L. fluviatilis* is represented graphically in Fig. 22 and Fig. 23 respectively. The figures show the reaction times obtained at the two highest and two lowest light intensities plotted in relation to the length class. The change in the reaction time occurring in two individuals of each species is shown in Fig. 24 for *L. planeri* and Fig. 25 for *L. fluviatilis*.

In animals shorter than 5 mm. the reaction times were long, varying over an approximate range of 20-100 sec. Above 5 mm., the reaction time first decreased sharply in relation to the linear length scale. The decrease became slower as the reaction time approached, at 6.5 mm., values similar to those obtained in older ammocoetes tested under the same conditions.

Although considerable variation occurred in the reaction times of animals less than 6.5 mm., the reaction times were on the whole shorter at the higher light intensities. In *L. planeri*, where
observations were begun at an earlier stage than in *L. fluviatilis*, reaction times appeared at the highest intensities earlier than at the lowest. In the graphs which combine the results from several animals, the effect of light intensity is illustrated by plotting the reaction times obtained at the extremities of the intensity range employed. Reaction times obtained at intermediate intensities generally lay between those obtained at the extremities. The graphs of individuals of *L. fluviatilis* show the position of reaction times obtained at an intermediate intensity. A clear relation between the reaction time and intensity for small intensity differences, illustrated by a reaction time-intensity graph, does not however occur until the animals have reached 6.5 mm.

2. The Change in the Frequency of the "No reaction."

The frequency of the "no reaction" from the commencement of tests until the "no reaction" disappeared is shown for *L. planeri* in Table 18. The frequency is expressed as the percentage of tests in which the "no reaction" occurred. The "no reaction" occurred in a high proportion of tests where the animals were shorter than 4.6 mm. Thereafter the proportion decreased until in animals longer than 5.6 mm., the "no reaction" was not obtained. The proportion of "no reactions" was on
the whole less at the highest light intensity range than at the lowest. The difference was most marked where the proportion decreased. There was a continuous decrease in the proportion at the highest intensity range, but at the lowest, the decrease did not begin until the animals reached 4.6 mm.

These experiments have shown that when the light response first appeared, long reaction times were intermixed with a high proportion of "no reactions." The proportion of "no reactions" decreased slightly in advance of the fall in the reaction time, giving rise to a period when the reaction times were of frequent occurrence yet remained long. Thereafter, the reaction time decreased while the "no reaction" disappeared.

These changes in the reaction time and the proportion of "no reactions" occur during a particular period of the animal's development. During this period, however, the length of the reaction time and the proportion of "no reactions" depend not only on the stage of development reached by the animal, but can be influenced by the light intensity at which they are measured.

The development of response in relation to time. The period during which the response to light develops has been described in relation to
the length of the animal. The approximate extent of the period in relation to a time scale is indicated in Figs. 26 and 27. The figures show a growth curve for each species. The main features of the development of the light response are marked on the curves.

The Light Response of Animals Reared in Darkness.

The gradual decrease in the reaction time was observed in animals which had been exposed to light both in the laboratory and during tests. It was therefore possible that the short reaction time developed as a result of repeated exposure to light, through the continued functioning of the receptors and nervous pathways involved in the response. This possibility was tested in the present experiment by measuring the reaction time of animals kept in darkness.

Animals were put into darkness at a stage of development shortly before the first response appeared in animals exposed to light. They were not again exposed to light until they were stimulated in the execution of a single set of tests. The results for three animals of L. planari are given in Table 19. The table shows the reaction times obtained at four intensities in each animal, and the order in which each test was made. For comparison, the results of a single set of tests at
the same intensities are shown for three animals which had been exposed to light. These animals were tested at approximately the same stage of development, which is indicated in all animals by their length.

Short reaction times were obtained from the animals kept in darkness and each set formed a reaction time-intensity curve. These results show that the short reaction time was not developed as a result of previous activity in the nervous pathways involved in the light response. The experiment was however only carried out, owing to loss of material, on animals kept in darkness during the period in which responses were obtained in other animals. It is possible therefore that earlier exposure to light involving activity which does not culminate in a motor response may influence the development of the initial long reaction times. The experiment does however show that the light stimuli to which the animals were repeatedly exposed in the previous investigation, do not promote the appearance of the fully developed response.

**The Development of Movement.**

This investigation was made to examine how far the development of the response to light
depended on the ability of the animals to move. The account of the development of movement is given from hatching onwards. It is compiled from observations made mostly on *L.fluviatilis*; both test animals and a number of stock animals were used. The observations were carried out on animals which had been disturbed either mechanically or by exposing them to light; the animals were subsequently measured. The following topics are considered:

1. The development of the swimming movement.

2. The movement occurring in response to a light stimulus.

3. The development of burrowing behaviour.

The account is summarised in Fig.28 where the stages in the development of each subject are shown in relation to the length of the animal; this enables a comparison to be made with the development of the light response.

1. The development of the swimming movement.

The period of development has been divided into three arbitrary stages. In the first, the movement of the animal does not result in progression. The second and third stages are distinguished by slow and fast progression respectively. In the non-progressive stage, the earliest movement is a single coil of the body to one side. This
can be a quick coil in which the body bends at one point and then relaxes, or a slow prolonged coil. In the quick coil the myotomes contract simultaneously, while in the prolonged coil slow serial contractions occur. Later, two successive coils on opposite sides of the body appear. The second coil begins before the first coil ends so that the body is thrown into an S-shaped curve. Again the contractions may be simultaneous or occur serially; in the former case, the body immediately assumes an S-shape and then relaxes, but in the latter, two separate coils can be distinguished. Gradually the successive coils become more frequent until two to three coils occur on each side of the body. This phase of the movement then passes into the second stage, namely the slow progressive movement, as the number of successive coils increases. Here, short bursts of activity occur in which it is possible to distinguish the individual coils. In the final stage, the full swimming movement is developed. Progression is fast and the activity is maintained over longer periods. The individual coils are indistinguishable owing to the increased rapidity of the contractions.

2. The movement occurring in response to a light stimulus.

The movement in its early stage is a slow coil
of the body to one side. This is followed by a short period when either a coil or a swimming movement occurs in response to light. Later, a swimming movement is the invariable response. Fig. 28 shows the range in length of the animal during which the coil occurs, and the length at which the swimming response is first observed.

3. The development of burrowing behaviour.

The first indication of the behaviour associated with an animal burying itself in sand is a swimming movement in which the head is directed downwards. This movement is not effective in penetrating sand and it is only later that sufficient thrust is developed to permit partial penetration. The development of burrowing behaviour is represented in Fig. 28 as two stages divided on the basis of this ability to penetrate into sand. When penetration has occurred, a burrowing movement beneath the sand accompanied by a slow lashing movement of the exposed tail, buries the remainder of the body.

Fig. 28 illustrates that the movement in response to light and the burrowing behaviour do not develop fully until the swimming movement has reached the fast progressive stage. The development of the swimming movement itself is a gradual process, in which the slow progressive movement and single and double coils can occur at the same stage.
of development. It has frequently been observed in dishes of animals brought into light that the first movements were isolated coils, and only later did the activity become continuous and the progressive movement appear. It is because the movement in response to light represents a first movement, that it remains a coil even after the animal is capable of swimming. The coil and hence the coil in response to light does not disappear until after the development of the efficient fast progressive swimming movement.

The change from the period of slow to fast progression is a gradual increase in the power of movement. The angled swimming movement, which is the preliminary movement in the burying of the animal, appears during the period of slow progression. The power of the swimming movement during this period does not allow the animal to penetrate the sand. Unless penetration occurs, the burrowing movement is not effective in burying the animal. Sufficient power to permit penetration is not acquired until the fast progressive swimming movement is well developed; only then is the burrowing behaviour complete.

The Development of Movement during the Period of Development of the Light Response. The non-progressive movement occurs before the first response
to light is obtained and continues during approximately the first half of the period where the reaction time is long. During the remainder of this period, the slow progressive movement occurs; the change to the fast progressive movement coincides with the appearance of the short reaction time. This means that when the response to light is fully developed, not only is the reaction time short but the movement of the response is fully developed: it is a swimming movement.

This study shows that the initial absence of a response to light is not due to the inability of the animal to move. While the light response is developing, however, changes are occurring the movement of the animal. These changes are an increase in the period during which movement is maintained and an increase in the rapidity of the serial contractions. Observations of the frequency of spontaneous movement have shown an increase in the frequency of such movement as the light response develops. These changes may be in some part associated with an increase in the excitability of the motor system. Such an increase could account for the changes in the reaction time and proportion of "no reactions" as the light response develops. Changes in the central nervous system as well as perhaps the incomplete development of the sensory system may account for the form of the developing light response.
Morphological Development.

The following account describes the main morphological changes that occur in the ammocoete from shortly before hatching until it becomes an active freeswimming larva. The account is given in relation to the length of the animal. It covers the period during which the development of the light response and the development of movement have been described. It provides information in addition to length as to the stage of development of the ammocoete during this period. Approximate time intervals before and after hatching are indicated, but these vary according to the temperature at which animals are reared.

The description is based on observations of specimens of *L. planeri* taken from two batches of animals reared at different times. Similar observations of *L. fluviatilis* do not reveal any differences between the two species, with regard to either the features described, or the length at which they appear. The development of *L. planeri* is illustrated in Fig. 29. The main stages in the development of *L. fluviatilis* are shown in Fig. 30.

**Length 2-3 mm.** At 2 mm., about four days before hatching, the head and tail of the animal curve towards the ventral surface. A white opaque appearance is due to the extensive presence of yolk
which forms a marked swelling in the posterior end, ten to eleven myotomes are visible (Fig. 29A). Gradually the anterior end straightens out and the posterior swelling is reduced. The heart becomes visible, beating slowly, and the limits of the yolk sac become apparent. A transparent fin appears extending from behind the head and continuing round the distended posterior region (Fig. 29B).

**Length 3-4 mm.** The anterior end of the animal becomes quite straight, but the posterior end remains curved downwards. The myotomes extend from a point behind the head to the beginning of the posterior curve. There is a gradual increase in transparency revealing the outline of axial structures and the yolk sac. The stomodaeeum is marked by a slit and anterior to this is a wide-mouthed pit (Fig. 29C). Hatching occurs towards the end of this stage and in the early part of the next.

**Length 4-5 mm.** The yolk sac is reduced and the posterior swelling is lost, though the tail retains a downward curve along which closely packed myotomes now extend. The dorsal and caudal fin broadens. Towards 5 mm. (Fig. 29D), the stomodaeeum becomes a deep transverse indentation while the opening to the pit anterior to it is reduced. Seven gill pouches are visible and at least the anterior ones have acquired external openings. The heart
acquires a regular beat.

**Length 5-6 mm.** By 5-5 mm. (Fig. 293), two days after hatching, the tail straightens but a slight downward curve is indicated by a faint curving of the myotomes which now reach the hind end of the yolk sac. An increase in transparency reveals the full extent of the yolk sac and its forward connection with the pharynx; the liver and outline of the endostyle are also visible. The stomodaeum is represented by a large cavity. Seven gill pouches acquire external openings shortly after 5 mm. Pigment is present in 5 mm. animals and is first deposited on either side of the midline, anterior and dorsal to the first gill slit. Later, pigment extends dorsally across the midline and vertically towards the first gill slit.

**Length 6-7 mm.** Early in this stage, the animal assumes a blunt nosed appearance due to the formation of the oral lips. The opening of the anterior pit is further reduced. The continuous fin in the caudal region becomes almost three times as broad as it is along the trunk. About 6-3 mm., the eye appears anterior to the pigmented area as a faintly pigmented oval. Later, the complete straightening of the tail is shown by the straightening of the posterior myotomes. The yolk, greatly reduced, lies in a swollen intestine which
at its hind end is arched and turned downwards to the outside. The velum is visible. The pigment is more extensive and is present in the snout, and in a line along the dorsal edge of the gills and the lower edge of the myotomes (Fig. 29P).

Length 7-8 mm. Morphological characters are very similar to those of the last stage. The pigment spreads ventrally from the original line over the yolk mass. Blood can be seen flowing in the ventral aorta. The stomodaeum opens during this stage, about six days after hatching.

The Development of the Delayed Response.

A marked delay in the reaction time was observed in older ammocoetes when both a light and thigmotactic stimulus were applied. The present experiment was designed to test whether the delay occurred in ammocoetes in which the response to light had newly developed.

As in the experiments on older ammocoetes, the reaction times obtained from animals when in contact with sand are compared with those obtained from animals lying in water only. Contact with sand was established by the method of pressing the animals against the sand by an overlying shallow layer of water. To ensure that these small animals were in contact with sand, the water layer was reduced until only the sinuous burrowing movement
occurred. Control tests were carried out in a shallow layer of water without sand.

A single set of tests was made on each animal at four light intensities when in contact with sand, followed by a similar set of control tests. If any reaction times from the control tests were unusually delayed, the results for that animal were rejected on the basis that they were affected by the adverse conditions of the experiment. The result from three animals were rejected for this reason. The experiment was performed with L. planerii.

Results.

The reaction times obtained from animals in contact with sand have been classed as "short" or "long" on the basis described in the water cell tests. The classification is based on the standard deviations of control reaction times obtained at the same intensity. For the calculation of these standard deviations at each intensity, reaction times from the control tests in this experiment have been combined with those of the control tests in the similar experiment where older ammocoetes were used.

The numbers of "short" and "long" reaction times obtained over the total intensity range are shown in Table 20. The data was obtained from eleven animals whose lengths ranged from 7.4 mm. to
10.0 mm. For comparison, similarly classified results from 44 tests carried out on older ammocoetes in contact with sand are presented. These results were picked at random from the identical experiment in which the same method of establishing contact with sand was used. 77% of the reaction times in young ammocoetes were "short," compared with only 16% in older ammocoetes. Only two of the "long" reaction times in young ammocoetes were longer than 20 sec. and the "no reaction" was absent.

No observations of the frequency of spontaneous movement were made for young ammocoetes lying on sand. It is unlikely, however, that the high proportion of "short" reaction times resulted from a high rate of spontaneous activity, since the "short" reaction times show a correlation with light intensity. The mean of the "short" reaction times at four light intensities are shown in Table 21.

The results show that delayed reaction times occurred less frequently in young ammocoetes than in older ammocoetes. Although animals move in response to the touch of a hair before the light response is fully developed, this does not necessarily indicate that the animal is receiving the thigmotactic stimulus used in this experiment; the stimulus differs from touch in that it is sustained contact applied to the greater part of the animals' body.
The rarity of the delayed reaction time in young ammocoetes may therefore be due to the incomplete development of the sensory system involved in the reception of the thigmotactic stimulus, as well as perhaps of a central nervous mechanism whose function would be to delay the response to light. The experiment indicates that the delayed response, resulting from the addition of a thigmotactic stimulus, develops independently of the response to a light stimulus alone.
A marked increase in the length of the reaction time has been observed when a thigmotactic stimulus is applied in addition to the light stimulus. The reaction time in response to a light stimulus alone is not itself constant but varies with the light intensity. The events which could account for the change in the reaction time at different intensities of the light stimulus are discussed first, since they lead to an explanation of the increase in the reaction time produced by a thigmotactic stimulus.

Studies on the anatomical basis of the light response in ammocoetes provide some information concerning the events which occur during the reaction time. Young (1935) showed that the tail was the region most sensitive to light, and that illumination of the tail produced a movement of the head when the spinal cord had been sectioned; the movement did not occur when the lateral line nerves had been cut. It may be concluded that impulses generated in supposed receptors are transmitted centrally along fibres running the lateral line nerves. The receptors themselves have not been conclusively identified. Steven (1951) has however described sensory cells most numerous in the epidermis of the tail which fit the requirements predicted for the
receptors concerned in the response. These cells are approximately 6 in diameter and bear bouton nerve endings on their surface; several cells are innervated by a single fibre. The spectral sensitivity of the ammocoete (Steven, 1950) indicates the presence of photosensitive pigment, probably porphyropsin, and would imply the existence of receptors in which the pigment is contained. These studies suggest that the response to light in the ammocoete involves the stimulation of photoreceptors which are innervated by lateral line fibres entering the central nervous system at the level of the medulla (Pearson, 1936). The reaction time can therefore be regarded as the time course of a series of events which begin with a primary photochemical reaction, leading, probably through coupling reactions (Wald, 1951), to the generation of impulses in the afferent nerves. Succeeding events would include the conduction of these impulses to the medulla, their central transmission and the subsequent generation of impulses in the motor nerves, resulting finally in muscular contractions. A change in the duration of one or more of these events must account for the change in the length of the reaction time.

In order to decide the events whose durations may vary, it is helpful to examine evidence concerning the changes which follow the illumination of
photoreceptors and the way in which light intensity affects these changes. The evidence has been obtained from studies on the arthropod and vertebrate eye using electrophysiological techniques. Illumination of photoreceptors is followed by a relatively long latent period* during which no electric changes can be detected. The first electrical change following the latent period is probably a depolarization of individual sense cells, such as has been demonstrated in the lateral eye of Limulus by intracellular recording (Hartline, Wagner and MacNichol, 1952); the depolarization is accompanied subsequently by impulses in the nerve fibre of the same cell. Wullf, Fry and Linde (1955) believe this depolarization to be the intracellular sign of the retinal action potential measured with extracellular electrodes; indeed, it had earlier been suggested (Hartline, 1935; Granit, 1947) that the retinal action potential was a generator potential initiating impulses in the optic nerves. Measurements have been made at different light intensities of the latency and magnitude of the retinal action potential (Wullf and Pandazi, 1951; Wullf et al., 1955), and the latency and magnitude of the discharge in optic fibres (Adrian and Matthews, 1927; Hartline, 1934

* This usage of the term latent period should be distinguished from the usage employed by Hecht (1918-19c).
and 1938). These measurements show that changes in the intensity of illumination affect both the latency and magnitude of the electrical response. At higher intensities, there is a decrease in the latency of the particular electrical event, an increase in the size of the retinal action potential and an increase in the frequency and total number of impulses in the optic nerves.

The inverse relation between the latency of electrical events and the light intensity suggests that the change in the reaction time at different intensities may be in some part due to a change in the duration of events which lead to the generation of impulses. Changes in the latency of the retinal action potential indicate a change in the time required for the initial depolarization at different intensities. It is probable also that a change in the time required for the appearance of impulses after the onset of depolarization contributes towards the change in the reaction time; this is suggested by a discrepancy between the extent of the change in the latency of the retinal action potential and that of the nerve impulse discharge over an equal intensity range. The latency of the retinal action potential in the ocelli of Limulus varies from 0.023 to 0.113 sec. over a respective intensity range of $2.7 \times 10^4$ to $2.7 \times 10^{-2}$ f.c. (Wullf and
Pandazi, 1951). The discharge of impulses from single receptors in a similar preparation, the lateral eye of *Limulus*, shows a greater variation in latency, 0.077 to 0.505 sec., over a smaller intensity range of approximately $3 \times 10^5$ to $3 \times 10^2$ f.c. (Hartline, 1934). The discrepancy could be accounted for by supposing that the interval between the onset of depolarization and the initiation of impulses depends on the magnitude of the depolarization; the interval would vary at different intensities (cf. Wullf and Pandazi, 1951).

The maximum change in the duration of events in the receptor will therefore be that shown by the latency of nerve impulse discharge, rather than the retinal action potential. Latencies of nerve impulse discharge should then indicate how far the change in the reaction time can be attributed to events in the receptor. Although no values are available for isolated photoreceptors such as occur in the ammocoete, it is unlikely that the latency of these receptors would differ markedly from that of optic receptors; there is at least reasonable agreement between the latencies of photoreceptors in the structurally different vertebrate and invertebrate eyes. Some values of the latency of nerve impulse discharge obtained from various preparations are shown in Table 22. The shortest latencies are those obtained from vertebrate preparations; this
might be expected in view of the opportunity for
the summation of subliminal electrical effects in
the vertebrate retina. Latency values from single
receptors in the lateral eye of Limulus (Hartline,
1934) probably come nearest to those of isolated
photoreceptors. They indicate a change in latency
of less than 0.5 sec. over a light intensity range
of 3 log. units. A change of this order would be
insufficient to account for the whole of the change
in the ammocoete. The reaction time varies from 1
to 3 sec. at moderate intensities ranging over 2 log.
units. When the intensity range is extended by a
further 3 log. units covering very low intensities,
the reaction time increases up to 20 sec. after
which movements in response to light become indis-
tinguishable from spontaneous movements. It is
probable that changes in the latency of the receptor
make a partial contribution towards the change in
the reaction time, the contribution being proportion-
ally greater where the reaction time is short than
where it is long.

Apart from the change in the reaction time at
different intensities, there is also the variability
of the reaction time at a given intensity to be
explained, a variability which increases at lower
intensities (cf. Obreshkove, 1921). There is no
evidence of an increased variability of the latent
period at lower intensities which might account for this.

The origin of the remainder of the change in the reaction time must be sought in the second effect of light intensity on the activity of photoreceptors, namely the effect on the magnitude of the electrical change. This effect is of course the well known mechanism which photoreceptors share with other sensory receptors (Matthews, 1931; Bronk and Stella, 1935; Pfaffman, 1941), whereby the intensity of the stimulus is signalled by the frequency of the nerve impulse discharge. It is possible that the frequency of discharge in the afferent nerves may influence the time required for the generation of impulses in the motor nerves. Neurophysiological studies on synaptic transmission in spinal reflexes (Eccles, 1953) have clearly established a basis for such a possibility. They show that the generation of a propagated impulse in a motor nerve depends on the summation of small electrical potentials to a critical level of depolarization. The small potentials are produced by presynaptic impulses and each decays exponentially in time. The potentials can only summate to a critical level when the interval between impulses arriving at a motor neurone is sufficiently short; with still shorter intervals the critical level is reached sooner.
It is of course only impulses in different presynaptic fibres that can arrive in a sufficiently short space of time. An increased frequency in the afferent discharge could be held to decrease the interval between impulses arriving at a motor neurone in two ways. It would first increase the frequency of impulses in any one internuncial neurone, and secondly increase the total number of active neurones by recruiting those of higher threshold. The reaction time may partly be the time required for the continued afferent discharge at each intensity to excite sufficient numbers of internuncial neurones for summation to occur at motor neurones and reach a critical level for the generation of impulses (cf. Lorente de Nó, 1938).

The effect of the size of the afferent discharge on the time required for the generation of impulses is evident from the shortening of central reflex time that occurs with an increased intensity of stimulus (Fulton, 1949). Where the discharge in motor nerves depends on activity in chains of internuncial neurones, the peak of the discharge is reached earlier at higher intensities of stimulus (Lorente de Nó, 1938). The extent of these temporal changes are however to be measured in milliseconds; there are no values available which indicate a change in the time required for the transmission of impulses.
occupying several seconds. Synaptic transmission in the central nervous system has, on the other hand, been studied mainly in spinal reflexes that involve relatively few neurones. The magnitude of the temporal changes so far observed might be extended in the brain with the greater number of neurones involved. The light response in the ammocoete is not a spinal reflex since the afferent nerves enter at the level of the medulla.

To account for part of the change in the reaction time at different intensities by variations in the time required for the central transmission of impulses would, at the same time, provide an understanding of the variability of the reaction time at a given intensity and the increase in this variability at lower intensities. The appearance of impulses in the motor nerves would depend on the activity of many internuncial neurones; the instant at which a critical level of depolarization is reached is likely to be more variable where the intensity of the afferent stimulus just meets the threshold of the motor nerves.

An indication that the reaction time in the ammocoete is partly dependent on the duration of events in the nervous system is given by the work of Young (1935). He reported a few observations showing that the reaction time decreased when the area of the body illuminated was increased. The
finding requires further investigation, for any such decrease must be attributed to spatial summation in the nervous system.

It is interesting to note, in connection with the suggested dependence of the reaction time on the frequency of nerve impulse discharge, that the frequency of the initial maximum discharge from single receptors in the eye of Limulus is linearly related to the logarithm of the light intensity (Hartline and Graham, 1932). If a similar relation held for the discharge from photoreceptors in the ammocoete, the reaction time would be roughly proportional to the frequency of discharge, since reaction time-intensity graphs are approximately linear when plotted in relation to the logarithm of the light intensity.

The existence of a delay of several seconds between the beginning of stimulation and a motor response has been observed in the light response of Ciona, Mya and Pholus (Hecht, 1918-19a,b and 1927-28), Rana tadpoles (Obreshkove, 1921), Proteus (Hawes, 1945) and Myxine (Newth and Ross, 1955; Steven, 1955). Sensitivity to light in these animals, as in the ammocoete, has been attributed to photoreceptors scattered in the skin. The dependence of the response on such receptors may be one reason why a reaction time of several seconds
occurs. The receptors may be relatively few in number and constitute a relatively small part of the total afferent supply of the animal. The afferent discharge from a small number of fibres might require some time to excite sufficient numbers of internuncial neurones for the impulses arriving at a motor neurone to summate.

The proposed composition of the reaction time and the events which contribute towards the change in its duration at different intensities is not however in accord with Hecht's (1918–19a,b,c,d, 1919–20a,b, and 1920–21) analysis of the reaction time in Ciona and Mya. The length of the reaction time in these animals is of the same order as that found for the ammocoete, and similarly varies inversely with the intensity of light. Hecht regarded the reaction time as the duration of chemical reactions taking place in the receptor; he supposed that a primary photochemical reaction was followed by a secondary non-photochemical reaction whose end product generated the discharge of impulses. His conclusions stated in terms of the later electrophisiological studies on photoreceptors (loc.cit.) are equivalent to supposing the latency of nerve impulse discharge to occupy almost the whole of the reaction time, and only a negligible interval to be required for the central
transmission of impulses. Hecht attributed the change in the reaction time at different intensities to a change in the duration of the photochemical reaction. This is equivalent to supposing the change in the reaction time to be entirely due to variations in the latency of discharge.

Hecht's view that the reaction time is the duration of events in the receptor is based on an empirical division of the reaction time into an initial sensitization period and subsequent latent period.* It depends on the demonstration that the sensitization period is the duration of a photochemical reaction and the latent period the duration of a secondary reaction. Determined experimentally, the sensitization period is the interval during which it is necessary to illuminate the animal in order to obtain the shortest possible reaction time at a given intensity. The shortest possible reaction time is associated with a maximal effect, namely a latent period of constant minimal duration. Assuming a constant latent period to represent a given effect, the sensitization period would be the duration of a photochemical reaction if its duration varied with intensity according to the Bunsen-Roscoe

* This usage of the term latent period should be distinguished from its more common usage referring to the interval between the application of a stimulus and the electrical response.
reciprocity law of photochemistry. This was, however, only demonstrated for Ciona (Hecht, 1918-19a). The sensitization period measured at various intensities was found to vary inversely with the intensity; the product of the sensitization period and the intensity at which it was measured was a constant, indicating the applicability of the Bunsen-Roscoe law. Similar determinations were not made for Mya. In Myxine (Newth and Ross, 1955), the product of the sensitization period and intensity decreases at lower intensities; there would therefore be no reason to suppose the sensitization period represents the duration of a photochemical reaction in this animal. Sensitization periods ranging from 0.5 to 9.5 sec. were obtained for Ciona (Hecht, 1918-19a), and from 5 to 42 sec. for Myxine (Newth and Ross, 1955). To suppose all but the shortest of such intervals to be the duration of events occurring in the receptor is incompatible with latency of discharge indicated by electrophysiological work. It is well known (Hartline, 1941) that the discharge of nerve impulses from photoreceptors continues during illumination; it is possible that the continued discharge can affect the length of the reaction time (cf. Lorente de Nó, 1938). Since the sensitization period is defined as the exposure required to produce the shortest
reaction time, the sensitization period might be the exposure required to produce the maximum duration of discharge that can influence the length of the reaction time.

Hecht (1919-20a) was unable to carry out determinations of the sensitization period at different intensities in *Mya*, owing to a technical difficulty arising from the shortness of the sensitization period. He did show, however, that the Bunsen-Roscoe law described the relation between exposure and intensity for the production of a given minimal effect. He measured the minimum intensity which just elicited a response (presumably the longest reaction time) for several different exposures of fixed duration. The product of the exposure and intensity was found to be constant. Hecht concluded that a photochemical reaction took place during the exposure time, which would therefore be the duration of an event in the receptor. His view is not however here incompatible with electrophysiological studies since he used exposures, 0.016 to 0.104 sec., lying within the range of the latency of impulse discharge. Hecht (1920-21) later showed for *Mya* that effects greater than the minimum were described by the equation:

\[ E = k t \log I \]

where \( E \) is any effect greater than the minimum,
t the exposure and I the intensity. It follows that the time taken to produce a maximum effect, namely the sensitization period, will not vary with intensity in the Bunsen-Roscoe manner.

The latent period is an interval during which illumination is not required in order to produce a response (Hecht, 1918-19a,c). Its duration is constant and minimal over a considerable range of intensity, provided the animal is illuminated for the whole of the sensitization period (Hecht, 1918-19a,b). Exposures shorter than the sensitization period do however lengthen the reaction time and therefore the latent period also; Hecht (1918-19a,c) argued from this observation that the process taking place during the latent period must occur in a locality where it can be affected by the amount of photochemical change produced during the exposure time. He assumed it to take place in the sense organ and regarded it as a secondary reaction catalysed by the products of the photochemical reaction. Hecht failed, however, to give consideration to the possibility that the amount of photochemical change can influence the duration of a process in a separate locality through nervous connections. Adrain and Matthews (1927) recognised such a possibility in their explanation of the relation between the size of the area illuminated
and the latency of the optic nerve discharge in the conger eel. Hecht's (1918-19d) demonstration of the thermal nature of the latent period does not necessarily support the assumption that it is the duration of an event in the receptor.

Hecht (1920-21) attributed the change in the length of the reaction time at different intensities entirely to a change in the duration of the sensitization period. The change in the reaction time is assumed to be due to the different time required at each intensity for the production of a fixed amount of photochemical change. If, however, allowing sufficiently long exposures, the discharge of impulses from a receptor were proceeded at any intensity by the same amount of photochemical change, it would be difficult to account for the difference in the magnitude of the impulse discharge (or generator potential) at different intensities (loc. cit.). It has in fact been shown (Hartline, 1934; Wulff et al., 1955) by using exposures less than the critical duration (Hartline, 1928) that a constant amount of photochemical change in the receptor is accompanied by an electrical response of constant magnitude. It has further been stated (Wulff et al., 1955) that the difference in the electrical response at different intensities, which occurs when exposures exceed the critical duration,
is due to the different amount of photochemical product formed at each intensity in an almost constant interval. Indeed, a requirement for intensity discrimination, which is no doubt related to the size of the discharge, would be a mechanism which compensated for the attainment of an equal photochemical change at different intensities by varying exposures. Where such a mechanism does not operate, as it does not for exposures less than the critical duration, intensity discrimination fails. Adrian and Matthews (1927) have already pointed out the significance of the work of McDougall (1904) on human vision in this connection. He found that intensity discrimination failed where very short flashes of light were presented to the subject; the brightness of the sensation depended solely on the total quantity of light received by the eye. This finding is understandable if the flashes were less than the critical duration; for such flashes, the size of the optic discharge depends not on the intensity but on the total energy content of the flash.

Hecht's explanation of the change in the reaction time would not account for the increased variability of the reaction time at a given intensity where the intensity is lower. The sensitization period would not be expected to
increase in variability simply where a longer time were required for a photochemical reaction to form a constant amount of product.

Hecht's reasons for believing that the reaction time almost exclusively represents the duration of events in the receptor have been criticised here. Newth and Ross (1955) have pointed out that his description of the sensitization process in Mya is inadequate for Ciona, the ammocoete and Myxine. Theories of dark adaptation of the type that Hecht (1918-192) derived from his analysis of the reaction time have been criticised by Granit (1955) and Pirenne (1956).

It has previously been suggested that only part of the reaction time occurs in the receptor and that the remainder is the time required for the afferent discharge to generate motor impulses. It has further been suggested that the length of the reaction time partly depends on the frequency of the afferent discharge. Long reaction times are obtained at very low light intensities and are mostly due to the longer time required for the lower frequency of discharge to excite the motor nerves. After the introduction of a thigmotactic stimulus, similarly long reaction times are obtained at moderate light intensities, and are therefore equivalent to the reaction times produced at a lower
frequency of discharge. In this respect, the effect of a thigmotactic stimulus resembles the effect of an inhibitory discharge on the activity of motor neurones as observed in spinal reflexes (Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932; Eccles, 1953). The discharge in a motor neurone subjected to both inhibitory and excitatory impulses is equivalent to that which would be obtained from a lowered intensity of the excitatory volley alone. The increase in the reaction time observed in the presence of a thigmotactic stimulus can therefore be explained by supposing the thigmotactic stimulus to produce an inhibitory discharge and the light stimulus an excitatory discharge. It is significant here that the reaction time in the presence of a thigmotactic stimulus retains a relation to the intensity of the light stimulus: the generation of impulses in motor nerves would remain dependent on the magnitude of the excitatory volley, since inhibition is a hyperpolarization of the surface membrane of the neurone and simply increases the extent of the depolarization required to generate an impulse (Eccles, 1953).

In the absence of information on the tactile sense in the ammonoete, the origin and site of action of the inhibitory effect of the thigmotactic stimulus remains uncertain. It is of interest,
however, that apart from the occasional occurrence of the "short" reaction time, the inhibition appears to be maintained as long as the animal is in contact with sand. This prolonged inhibition could arise from a continuous inhibitory bombardment of the motor neurones due to activity in chains of inter-neurones, maintained by a tonic discharge from the touch receptors (cf. Lorente de No, 1938). Oscillations in activity might account for the occasional "short" reaction time.

The effect of a thigmotactic stimulus was measured in the present experiments as an increase in the reaction time in response to a light stimulus. Francis and Horton's (1936) observations of thigmotaxis in the ammocoete can be interpreted as a decrease in the amount of locomotory activity. Jones (1955) direct measurements of activity confirm this interpretation. He found that locomotory activity in ammocoetes was reduced when small glass tubes were added to the experimental tank which otherwise contained only water; the animals were unable to establish contact with the glass tubes. Activity was reduced both in darkness and during illumination. There was however an indication that activity was slightly more frequent in animals exposed to light. The experiments isolated the effect of a thigmotactic stimulus from that produced
by a light and thigmotactic stimulus combined. They suggest that the thigmotactic stimulus inhibits motor activity and that the excitatory effect of light is superimposed on this inhibition. The observations are in accord with the observed increase in the reaction time and are consistent with the conception of the inhibitory nature of a thigmotactic stimulus.

Reaction times similar to those obtained after the introduction of a thigmotactic stimulus have also been obtained over a similar range of light intensity during the early development of the response. It would be possible to account for the long reaction times that occur during early development on the earlier suggestion that the reaction time is partly the time required for afferent impulses to excite sufficient numbers of internuncial neurones for spatial summation to be effective in generating motor impulses. At an early stage of development, there may be few functional receptors and perhaps a lack of internuncial connections in the central nervous system. There are, at this time, changes in the movement of the animal which suggest that new nervous connections are being established (Coghill, 1929). Few functional receptors would be equivalent to a lowered frequency of afferent discharge, while lack of internuncial
connections would decrease the opportunity for spatial summation.

The form of the developing light response can be understood as a gradual increase in the magnitude of the afferent discharge and or the total number of active internuncial neurones. As would be expected, the gradual disappearance of the "no reaction" and the changes in the reaction time occur at highest light intensities in advance of the same changes at lower intensities, and may be attributed to the greater excitatory discharge at higher intensities.

The work has been concerned with a delay between the beginning of stimulation and a motor response. The delay has been partly attributed to the time required for the excitation of the motor nerves. As far as the behaviour of the animal is concerned, the delay signifies the power of the stimulus to excite and the ability of the motor nerves to be excited. In this respect the delay represents the intensity of the response, since in situations where more than one type of response can occur the response most readily excited, that having the shortest reaction time, will predominate.

The response to light was greatly reduced in the presence of a thigmotactic stimulus. It nevertheless remained dependent on the intensity of
the light stimulus. To a certain extent, the effect of a thigmotactic stimulus was compensated for by an increase in the intensity of the light stimulus. Where afferent impulses from different sensory receptors compete for the control of the motor neurone, both the sensory field from which the impulses originate and their frequency determine the production of a given response. The work has shown how the fundamental properties of the neurone derived from studies of the neurone in isolation can be applied to account for a change in the behaviour of an animal.
I wish to thank Professor D. M. Steven for much helpful advice and encouragement throughout this work, and Professor M. M. Swann for his kindness in reading the manuscript. I also wish to thank Dr. L. J. Hale for invaluable assistance with statistical problems. I am deeply indebted to Mr. R. A. Fox for preparing all the photographic prints, and Mr. E. A. Lucey who took some of the films.
REFERENCES.


Hartline, H. K. 1941. The neural mechanisms of vision. Harvey Lectures, 39-68.


Table 1. The mean and standard deviation of the reaction time at different light intensities.

The calculation was made from 20 reaction times at each intensity; equal numbers were obtained by random selection; data from 6 animals.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Mean reaction time (sec.)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.072</td>
<td>0.78</td>
<td>0.13</td>
</tr>
<tr>
<td>1.680</td>
<td>1.33</td>
<td>0.32</td>
</tr>
<tr>
<td>1.041</td>
<td>1.59</td>
<td>0.37</td>
</tr>
<tr>
<td>0.654</td>
<td>2.17</td>
<td>0.88</td>
</tr>
<tr>
<td>0.072</td>
<td>2.89</td>
<td>1.86</td>
</tr>
</tbody>
</table>
Table 2. The frequency of the "no reaction" at different light intensities during successive tests.

The frequency is shown as a percentage of the total number of tests made; day 1 represents the first day on which the animal was tested; successive tests at intervals thereafter; data from 6 animals.

<table>
<thead>
<tr>
<th>Time of tests (days)</th>
<th>1</th>
<th>5 and 6</th>
<th>11 and 13</th>
<th>15 and 16</th>
<th>Total period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity range (log.of f.c.)</td>
<td>No. of NR*</td>
<td>Total No. NR* (%)</td>
<td>No. of NR*</td>
<td>Total No. NR* (%)</td>
<td>No. of NR*</td>
</tr>
<tr>
<td>0.881-1.362</td>
<td>4</td>
<td>26</td>
<td>15</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>0.602-1.176</td>
<td>3</td>
<td>27</td>
<td>11</td>
<td>34</td>
<td>47</td>
</tr>
<tr>
<td>1.602-3.01</td>
<td>21</td>
<td>37</td>
<td>57</td>
<td>45</td>
<td>73</td>
</tr>
<tr>
<td>Temperature range during tests (°C)</td>
<td>15.2-19.5</td>
<td>14.0-18.8</td>
<td>15.3-20.0</td>
<td>13.3-20.8</td>
<td>13.3-20.8</td>
</tr>
</tbody>
</table>

* "No reaction."
Table 3. Results of water cell experiment.

The reaction times and number of "no reactions" at 4 light intensity classes for animals buried in the water cell.

<table>
<thead>
<tr>
<th>Intensity (log.of f.c.)</th>
<th>Reaction times (sec.)</th>
<th>No. of NR</th>
<th>Total No. of tests</th>
<th>NR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.361</td>
<td>0.8 6.2 12.7 14.4 17.0 18.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.273</td>
<td>0.9 9.4 13.2 16.0 17.4</td>
<td>11</td>
<td>27</td>
<td>41</td>
</tr>
<tr>
<td>1.268</td>
<td>1.0 11.8 14.2 16.7 17.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.889</td>
<td>0.7 0.9 6.3 14.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.810</td>
<td>0.7 0.9 10.6 14.8</td>
<td>18</td>
<td>29</td>
<td>62</td>
</tr>
<tr>
<td>0.747</td>
<td>0.7 2.4 14.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.368</td>
<td>0.7 3.5 9.7 14.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.268</td>
<td>0.9 3.8 12.7 15.5</td>
<td>17</td>
<td>29</td>
<td>59</td>
</tr>
<tr>
<td>0.264</td>
<td>3.2 6.5 13.4 15.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.813</td>
<td>0.9 1.7 6.5 19.3</td>
<td>19</td>
<td>29</td>
<td>66</td>
</tr>
<tr>
<td>1.736</td>
<td>1.1 6.0 9.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.683</td>
<td>1.5 6.1 9.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>65 114</td>
<td>57</td>
</tr>
</tbody>
</table>

Temperature range during tests (°C) 18.3 - 19.5
Table 4. Results of water cell experiment.

The mean and standard deviation of reaction times from the control tests at 4 light intensity classes; intensity values were similar to those of Table 3.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Mean reaction time (sec.)</th>
<th>Standard deviation</th>
<th>No. of tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.447</td>
<td>1.36</td>
<td>0.89</td>
<td>57</td>
</tr>
<tr>
<td>1.332</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.934</td>
<td>1.94</td>
<td>0.69</td>
<td>25</td>
</tr>
<tr>
<td>0.819</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.423</td>
<td>2.44</td>
<td>1.31</td>
<td>54</td>
</tr>
<tr>
<td>0.320</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.924</td>
<td>3.45</td>
<td>1.70</td>
<td>20</td>
</tr>
<tr>
<td>1.813</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temperature range during tests (°C) | 15.0 - 18.5
Table 5: Results of water cell experiment.

The numbers of "short" and "long" reaction times and "no reactions" obtained in the buried tests; the combined percentage of "long" reaction times and "no reactions" represents the proportion of results differing from the controls; the upper limit of the "short" reaction time was calculated from data given in Table 4.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Upper limit of &quot;short&quot; RT* (sec.)</th>
<th>No. of &quot;short&quot; RT*</th>
<th>No. of &quot;long&quot; RT*</th>
<th>No. of NR</th>
<th>Total No. of tests</th>
<th>&quot;Long&quot; RT* + NR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.361</td>
<td></td>
<td>3</td>
<td>13</td>
<td>11</td>
<td>27</td>
<td>89</td>
</tr>
<tr>
<td>1.273</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.889</td>
<td></td>
<td>6</td>
<td>5</td>
<td>18</td>
<td>29</td>
<td>79</td>
</tr>
<tr>
<td>0.810</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.747</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.368</td>
<td></td>
<td>5</td>
<td>7</td>
<td>17</td>
<td>29</td>
<td>83</td>
</tr>
<tr>
<td>0.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.264</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.813</td>
<td></td>
<td>7</td>
<td>3</td>
<td>19</td>
<td>29</td>
<td>76</td>
</tr>
<tr>
<td>1.736</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.683</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>21</td>
<td>28</td>
<td>65</td>
<td>114</td>
<td>82</td>
</tr>
</tbody>
</table>

* Reaction time.
Table 6. Results of wet sand experiment.

The mean and standard deviation of control reaction times and the calculated upper limit of the "short" reaction time for 8 light intensity classes; the distribution into "short" and "long" reaction times and "no reactions" of the results of sand tests for the same intensity classes; the combined percentage of "long" reaction times and "no reactions" represents the proportion of sand tests wherein the result differed from that of the controls.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Control tests</th>
<th>Sand tests</th>
<th>&quot;Long&quot; RT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RT (sec.)</td>
<td>S.D.* No. of tests</td>
<td>Upper limit of &quot;short&quot; RT (sec.)</td>
</tr>
<tr>
<td>1.966</td>
<td>1.01 0.21</td>
<td>14</td>
<td>1.46</td>
</tr>
<tr>
<td>1.683</td>
<td>1.10 0.60</td>
<td>11</td>
<td>2.42</td>
</tr>
<tr>
<td>1.518</td>
<td>1.41 0.33</td>
<td>23</td>
<td>2.09</td>
</tr>
<tr>
<td>1.470</td>
<td>1.212</td>
<td>1.43 0.44</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>0.982</td>
<td>1.93 0.55</td>
<td>3.27</td>
</tr>
<tr>
<td></td>
<td>0.683</td>
<td>1.83 0.51</td>
<td>2.97</td>
</tr>
<tr>
<td>0.506</td>
<td>0.447</td>
<td>3.40 1.56</td>
<td>6.66</td>
</tr>
<tr>
<td>0.190</td>
<td>1.960</td>
<td>3.02 2.79</td>
<td>8.85</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temperature range during tests (°C) 13.8 - 17.9

* Standard deviation.
Table 7. Results of wet perspex experiment.

The mean and standard deviation of control reaction times and the upper limit of the "short" reaction time for 3 light intensities; the distribution into "short" and "long" reaction times and "no reactions" of the results of perspex tests at the same intensities; the combined percentage of "long" reaction times and "no reactions" represents the proportion of perspex tests wherein the results differed from that of the controls.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Control tests</th>
<th>Perspex tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RT (sec.)</td>
<td>S.D.</td>
</tr>
<tr>
<td>2.152</td>
<td>0.99</td>
<td>0.22</td>
</tr>
<tr>
<td>1.939</td>
<td>0.96</td>
<td>0.24</td>
</tr>
<tr>
<td>1.703</td>
<td>1.41</td>
<td>1.03</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temperature range during tests (°C) | 13.8 - 17.0 | 15.2 - 17.0
Table 8. Results of head buried experiment.

The numbers of "short" and "long" reaction times in the head buried tests for 9 light intensities; the mean and standard deviation of control reaction times and the upper limit of the "short" reaction time for the same intensities.

<table>
<thead>
<tr>
<th>Intensity (log of f.c.)</th>
<th>Control tests</th>
<th>Head buried tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean RT (sec.)</td>
<td>S.D.</td>
</tr>
<tr>
<td>2.057</td>
<td>1.00</td>
<td>0.33</td>
</tr>
<tr>
<td>1.857</td>
<td>1.52</td>
<td>0.27</td>
</tr>
<tr>
<td>1.650</td>
<td>1.64</td>
<td>0.52</td>
</tr>
<tr>
<td>1.568</td>
<td>1.79</td>
<td>0.19</td>
</tr>
<tr>
<td>1.371</td>
<td>2.01</td>
<td>0.75</td>
</tr>
<tr>
<td>1.170</td>
<td>2.47</td>
<td>0.72</td>
</tr>
<tr>
<td>1.037</td>
<td>2.10</td>
<td>0.87</td>
</tr>
<tr>
<td>0.826</td>
<td>2.80</td>
<td>0.78</td>
</tr>
<tr>
<td>0.664</td>
<td>2.70</td>
<td>1.34</td>
</tr>
<tr>
<td>0.528</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.328</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.037</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Temp. range during tests (°C) | 13.0 - 15.0 | 13.0 - 15.0
Table 9. Results of head buried experiment.

The total number of "no reactions" in the head buried tests and control tests for the whole intensity range.

<table>
<thead>
<tr>
<th></th>
<th>Control tests</th>
<th>Head buried tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of NR</td>
<td>56</td>
<td>74</td>
</tr>
<tr>
<td>Total No. of tests</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>Intensity range (log. of f.c.)</td>
<td>1.613 - 2.057</td>
<td></td>
</tr>
</tbody>
</table>
Table 10. Results of tail buried experiment.

The numbers of "short" and "long" reaction times and "no reactions" in the tail buried tests for 4 light intensities; the combined percentage of "long" reaction times and "no reactions" represents the proportion of tests wherein the result differed from that of the controls; details of control tests in Tables 4 and 5.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>No. of &quot;short&quot; RT</th>
<th>No. of &quot;long&quot; RT</th>
<th>No. of NR</th>
<th>Total No. of tests</th>
<th>&quot;Long&quot; RT + NR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.361</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>15</td>
<td>87</td>
</tr>
<tr>
<td>1.273</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.889</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>71</td>
</tr>
<tr>
<td>0.810</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.747</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.368</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>13</td>
<td>85</td>
</tr>
<tr>
<td>0.268</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.264</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.813</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>69</td>
</tr>
<tr>
<td>1.736</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.683</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>12</td>
<td>13</td>
<td>30</td>
<td>55</td>
<td>78</td>
</tr>
</tbody>
</table>
Table 11. The proportion of "reactions" at a single light intensity using sands of different particle sizes.

<table>
<thead>
<tr>
<th>Mean Particle Size (μ)</th>
<th>Sand tests</th>
<th>Control tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>425</td>
<td>214</td>
</tr>
<tr>
<td>No. of &quot;reactions&quot;</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Total no. of tests</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>&quot;Reactions&quot; (%)</td>
<td>47</td>
<td>62</td>
</tr>
<tr>
<td>Temp. range during tests (°C)</td>
<td>16.1-17.5</td>
<td>15.0-18.3</td>
</tr>
</tbody>
</table>
Table 12. The proportion of "reactions" at different light intensities in the absence of a contact stimulus.

<table>
<thead>
<tr>
<th>Mean intensity (log. of f.c.)</th>
<th>No. of &quot;reactions&quot;</th>
<th>Total No. of tests</th>
<th>&quot;Reactions&quot; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.007</td>
<td>10</td>
<td>11</td>
<td>91</td>
</tr>
<tr>
<td>2.513</td>
<td>14</td>
<td>17</td>
<td>82</td>
</tr>
<tr>
<td>2.012</td>
<td>11</td>
<td>18</td>
<td>61</td>
</tr>
<tr>
<td>3.515</td>
<td>8</td>
<td>18</td>
<td>44</td>
</tr>
<tr>
<td>3.013</td>
<td>3</td>
<td>18</td>
<td>17</td>
</tr>
</tbody>
</table>

Temp. range during tests (°C) 15.0 - 17.8
Table 13. The proportion of "reactions" at different light intensities in the presence of a contact stimulus.

<table>
<thead>
<tr>
<th>Mean intensity (log. of f.c.)</th>
<th>No. of &quot;reactions&quot;</th>
<th>Total No. of tests</th>
<th>&quot;Reactions&quot; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.513</td>
<td>17</td>
<td>23</td>
<td>74</td>
</tr>
<tr>
<td>2.093</td>
<td>18</td>
<td>26</td>
<td>69</td>
</tr>
<tr>
<td>1.300</td>
<td>16</td>
<td>27</td>
<td>59</td>
</tr>
<tr>
<td>0.805</td>
<td>11</td>
<td>29</td>
<td>38</td>
</tr>
<tr>
<td>0.300</td>
<td>12</td>
<td>29</td>
<td>41</td>
</tr>
<tr>
<td>1.744</td>
<td>10</td>
<td>29</td>
<td>34</td>
</tr>
</tbody>
</table>
Table 14. The proportion of "reactions" for stimuli of different duration.

<table>
<thead>
<tr>
<th>Duration of stimulus (sec.)</th>
<th>1</th>
<th>5</th>
<th>Continuous illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of &quot;reactions&quot;</td>
<td>4</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Total no. of tests</td>
<td>21</td>
<td>30</td>
<td>23</td>
</tr>
<tr>
<td>&quot;Reactions&quot; (%)</td>
<td>19</td>
<td>47</td>
<td>74</td>
</tr>
<tr>
<td>Intensity (log.of f.c.)</td>
<td>2.443</td>
<td>2.443</td>
<td>2.513</td>
</tr>
<tr>
<td>Temp. range during tests (°C)</td>
<td>18.3-19.3</td>
<td>17.5-19.2</td>
<td>15.8-19.0</td>
</tr>
</tbody>
</table>
Table 15. The maximum length of the reaction time regarded as a response to light for three periods during the development of the response in *L. planeri* and *L. fluviatilis*.

The table also shows the probability of occurrence of 1 or more spontaneous movements, calculated from the Poisson sum, during an interval equal to the maximum length of the reaction time; the number of movements observed in a given time from which calculations were made; the percentage of reaction times rejected on the basis of their representing a spontaneous movement.

<table>
<thead>
<tr>
<th></th>
<th><em>L. planeri</em></th>
<th></th>
<th><em>L. fluviatilis</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>Length (mm.)</td>
<td>2.45-4.5</td>
<td>4.6-5.8</td>
<td>5.9+</td>
<td>3.8-4.2</td>
</tr>
<tr>
<td>Maximum length of RT regarded as response to light (sec.)</td>
<td>99</td>
<td>58</td>
<td>19</td>
<td>$\infty$</td>
</tr>
<tr>
<td>Probability of spontaneous movement (%)</td>
<td>4.9</td>
<td>4.9</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>Total observation period (min.)</td>
<td>99</td>
<td>96</td>
<td>81</td>
<td>18</td>
</tr>
<tr>
<td>Total No. of movements</td>
<td>3</td>
<td>5</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Reaction times rejected (%)</td>
<td>6</td>
<td>18</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Length (mm.)</td>
<td>2.0-3.0</td>
<td>3.1-4.0</td>
<td>4.1-4.6</td>
<td>4.7-4.8</td>
</tr>
<tr>
<td>-------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Intensity (log. of I.C.)</td>
<td>No. of NR</td>
<td>RT (sec.)</td>
<td>No. of NR</td>
<td>RT (sec.)</td>
</tr>
<tr>
<td>2.148</td>
<td>3</td>
<td>65.0</td>
<td>3</td>
<td>66.5</td>
</tr>
<tr>
<td>1.907</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>1.719</td>
<td>3</td>
<td>-</td>
<td>4</td>
<td>84.7</td>
</tr>
<tr>
<td>1.665</td>
<td>4</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>1.417</td>
<td>3</td>
<td>26.4</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>1.199</td>
<td>3</td>
<td>99.3</td>
<td>96.7</td>
<td>5</td>
</tr>
<tr>
<td>1.117</td>
<td>4</td>
<td>-</td>
<td>3</td>
<td>30.0</td>
</tr>
<tr>
<td>0.863</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>0.684</td>
<td>4</td>
<td>92.2</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>0.642</td>
<td>2</td>
<td>57.9</td>
<td>99.0</td>
<td>4</td>
</tr>
<tr>
<td>0.396</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>0.179</td>
<td>4</td>
<td>5</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 17. The reaction time during the development of the light response for *L.fluviatilis*.

Single measurements of the reaction time and the number of "no reactions" are shown at 15 light intensities in relation to length; the "no reaction" was absent after 5.0 mm.; the mean and standard deviation of the reaction time is shown for lengths above 7.8 mm.; data from 12 animals; temperature range during tests was 14.3–18.5 °C.
<table>
<thead>
<tr>
<th>Intensity (log. of I glamorous)</th>
<th>No. of RT</th>
<th>&lt;1.860</th>
<th>1.084</th>
<th>4.75.0</th>
<th>6.4.6</th>
<th>7.8.0</th>
<th>6.4.6</th>
<th>6.7.7</th>
<th>7.8.9.7</th>
<th>Mean RT</th>
<th>S.D.</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,860</td>
<td>2</td>
<td>19.2</td>
<td>21.0</td>
<td>18.4</td>
<td>21.7</td>
<td>20.5</td>
<td>19.0</td>
<td>20.0</td>
<td>10.5</td>
<td>1.6</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>4.75.0</td>
<td>2</td>
<td>26.6</td>
<td>23.8</td>
<td>20.7</td>
<td>21.3</td>
<td>16.5</td>
<td>27.4</td>
<td>32.4</td>
<td>25.9</td>
<td>4.0</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>6.4.6</td>
<td>2</td>
<td>26.0</td>
<td>20.5</td>
<td>14.5</td>
<td>16.4</td>
<td>6.7</td>
<td>1.5</td>
<td>1.6</td>
<td>2.8</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>6.7.7</td>
<td>2</td>
<td>26.6</td>
<td>23.8</td>
<td>20.7</td>
<td>21.3</td>
<td>16.5</td>
<td>27.4</td>
<td>32.4</td>
<td>25.9</td>
<td>4.0</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>7.8.9.7</td>
<td>2</td>
<td>26.0</td>
<td>20.5</td>
<td>14.5</td>
<td>16.4</td>
<td>6.7</td>
<td>1.5</td>
<td>1.6</td>
<td>2.8</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Reaction Times = RT
Table 18. The change in the frequency of the "no reaction" during the development of the light response for *L. planeri*.

The frequency is given as a percentage of the total number of tests made; it is shown for 3 light intensity ranges and for the total range, during 5 stages of development designated by length.

<table>
<thead>
<tr>
<th>Length class (mm.)</th>
<th>2-3</th>
<th>3.1-4.0</th>
<th>4.1-4.6</th>
<th>4.7-5.2</th>
<th>5.3-5.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity range (log.of f.c.)</td>
<td>1.665-2.148</td>
<td>0.863-1.417</td>
<td>0.179-0.684</td>
<td>0.179-2.148</td>
<td></td>
</tr>
<tr>
<td>No.of NR tests</td>
<td>13</td>
<td>14</td>
<td>17</td>
<td>13</td>
<td>40</td>
</tr>
<tr>
<td>Total No.of tests</td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>No.of NR (%)</td>
<td>93</td>
<td>82</td>
<td>81</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>No.of NR</td>
<td>1</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>32</td>
</tr>
<tr>
<td>Total No.of tests</td>
<td>8</td>
<td>79</td>
<td>12</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td>No.of NR (%)</td>
<td>0</td>
<td>9</td>
<td>11</td>
<td>56</td>
<td>23</td>
</tr>
<tr>
<td>No.of (%) NR</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total (%) NR</td>
<td>9</td>
<td>12</td>
<td>50</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>
Table 19. Reaction time-intensity data from 3 animals of *L. planerri* kept in darkness during the development of the light response.

Similar data is presented for comparison from 3 animals exposed to light; stage of development indicated by length.

<table>
<thead>
<tr>
<th>Intensity (log. of f.c.)</th>
<th>Order of tests</th>
<th>Reaction time (sec.)</th>
<th>Intensity (log. of f.c.)</th>
<th>Order of tests</th>
<th>Reaction time (sec.)</th>
<th>Intensity (log. of f.c.)</th>
<th>Order of tests</th>
<th>Reaction time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal not exposed to light</td>
<td>Animal exposed to light</td>
<td>Animal not exposed to light</td>
<td>Animal exposed to light</td>
<td>Animal not exposed to light</td>
<td>Animal exposed to light</td>
<td>Animal not exposed to light</td>
<td>Animal exposed to light</td>
</tr>
<tr>
<td>2.148</td>
<td>4th</td>
<td>0.6</td>
<td>0.9</td>
<td>1.907</td>
<td>4th</td>
<td>0.8</td>
<td>0.9</td>
<td>1.719</td>
</tr>
<tr>
<td>1.665</td>
<td>2nd</td>
<td>0.7</td>
<td>1.6</td>
<td>1.417</td>
<td>2nd</td>
<td>0.8</td>
<td>1.7</td>
<td>1.199</td>
</tr>
<tr>
<td>1.117</td>
<td>3rd</td>
<td>0.9</td>
<td>1.3</td>
<td>0.863</td>
<td>3rd</td>
<td>1.1</td>
<td>1.7</td>
<td>0.684</td>
</tr>
<tr>
<td>0.643</td>
<td>1st</td>
<td>1.1</td>
<td>1.7</td>
<td>0.396</td>
<td>1st</td>
<td>1.6</td>
<td>2.1</td>
<td>0.179</td>
</tr>
<tr>
<td>Length (mm.)</td>
<td>7.3</td>
<td>7.4</td>
<td>7.3</td>
<td>7.8</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
</tr>
</tbody>
</table>


Table 20. The numbers of "short" and "long" reaction times and "no reactions" for young and older ammocoetes.

<table>
<thead>
<tr>
<th>Length (mm.)</th>
<th>Intensity range (log. of f.c.)</th>
<th>No. of &quot;short&quot; RT</th>
<th>No. of &quot;long&quot; RT</th>
<th>No. of NR</th>
<th>Total No. of tests</th>
<th>&quot;Long&quot; RT+NR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4-10.0</td>
<td>0.179-2.148</td>
<td>34</td>
<td>10</td>
<td>0</td>
<td>44</td>
<td>23</td>
</tr>
<tr>
<td>c.30</td>
<td>1.964-1.966</td>
<td>7</td>
<td>10</td>
<td>27</td>
<td>44</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 21. The mean of the "short" reaction times at different light intensities for young ammocoetes.

<table>
<thead>
<tr>
<th>Mean Intensity (log. of f.c.)</th>
<th>0.288</th>
<th>0.730</th>
<th>1.244</th>
<th>1.860</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Reaction Time (sec.)</td>
<td>1.87</td>
<td>1.75</td>
<td>1.56</td>
<td>1.31</td>
</tr>
<tr>
<td>No. of observations</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 22. The range in the latency of nerve impulse discharge at different light intensities in some invertebrate and vertebrate preparations.

<table>
<thead>
<tr>
<th>Author</th>
<th>Preparation</th>
<th>Latency range (sec.)</th>
<th>Intensity range</th>
<th>Difference in latency (sec.)</th>
<th>Relative intensity range (log. units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hartline (1934)</td>
<td>Single fibres from lateral eye of Limulus</td>
<td>0.077-0.505</td>
<td>3.0x10^6-3.0x10^3 metre candles at surface of eye</td>
<td>0.428</td>
<td>3</td>
</tr>
<tr>
<td>Adrian and Matthews (1927)</td>
<td>Optic nerve of conger eel</td>
<td>0.15-0.35</td>
<td>40-2.5 arbitrary units</td>
<td>0.20</td>
<td>1.2</td>
</tr>
<tr>
<td>Hartline (1938)</td>
<td>Single intra-ocular fibres from frog retina</td>
<td>0.05-0.2</td>
<td>2.0x10^3-2.0x10^-2 metre candles at retina</td>
<td>0.150</td>
<td>5</td>
</tr>
<tr>
<td>Bernhard (1940)</td>
<td>Optic fibres of frog</td>
<td>0.100-0.400</td>
<td>10^0 x 10^-4 arbitrary units</td>
<td>0.300</td>
<td>4</td>
</tr>
<tr>
<td>Bernhard (1940)</td>
<td>Human electroretinogram. Latency of b-wave*</td>
<td>0.058-0.121</td>
<td>10^0 x 10^-3 arbitrary units</td>
<td>0.063</td>
<td>3</td>
</tr>
</tbody>
</table>

* The b-wave signals activity in the neurones linking the receptors and ganglion cells (Granit, 1947).
Fig. 1. Photographs of the apparatus designed to provide a light stimulus of variable intensity and duration for use with three animals.
Fig. 2. Diagram of the apparatus for light stimulation. B1 and B2, baffles; C, camera shutter; D, test dish; F1, ON 20 Chance glass heat filter; F2, monochromatic Ilford filter No.609; L1 and L2, convex lenses; M1, M2 and M3, positions of the plane mirror; OL, observation lamp; R, rheostat; S, light source; Sc, screen surrounding the three experimental positions I, II, III; T, transformer; W, neutral wedge of graded density.
Fig. 3. Reaction time-intensity graph: the relation between the reaction time and the logarithm of the light intensity. The graph is plotted from the mean reaction times given in Table 1.
Fig. 4. Reaction time–intensity graphs for two ammocoetes. Open circles Animal A, full circles Animal B. Small circles represent single measurements of the reaction time, the nine reaction times plotted at each intensity were selected at random. Large circles show the mean reaction time at each intensity.
Fig. 5. The frequency of the "no reaction" at successive tests. The graph is plotted from data given in Table 2. Time of tests in days after the first test (day 1). The frequency is plotted for three ranges of light intensity, 0.4-2.0 f.c. (open circles), 4-15 f.c. (half circles) and 23-76 f.c. (full circles).
Fig. 6. The relation between the reaction time and temperature for two light intensities. Open circles 1.03 f.c., full circles 118 f.c. Each point represents a single measurement of the reaction time.
Fig. 7. The water cell for testing ammocoetes buried in sand. The sectional width of the cell was reduced by a perspex block (PB) and glass plates (GP), so that an animal (A) buried in the sand (S) lay with part of its body exposed against the face of the cell. WL, water level.
Fig. 8. Modification of the apparatus for light stimulation used in the water cell tests. The water cell (WC) was mounted in the path of a horizontal beam of light emerging from the convex lens (L2). The first experimental position (I) was used for control tests by interposing the plane mirror at M1. C, camera shutter; D, test dish; P, mounting plate. Remainder of apparatus as in Fig. 2.
Fig. 9. The light intensity at the midpoint of the sand for two water cells:

A. Where the reduction in intensity is entirely due to the sand (S).

B. Where the reduction in intensity is partly due to the sand and partly due to the glass plates and perspex block (G&P).

The light intensity at different points in the cells is shown as a fraction of the incident light intensity (i). The intensity of light (t) which has passed through the whole cell is assumed for argument to be reduced to 1/8 of the incident light intensity. In cell B, the reduction in intensity due to the sand is less than in cell A. The intensity at the midpoint (M) of the sand in cell B is therefore higher than that in cell A.
Fig. 10. Crystallizing dish and fitment for testing ammocoetes with the anterior part of the body buried in sand. The dish was fitted with a central perspex block (PB) bearing a number of transverse partitions (TP) which divided the space (S) to be filled with sand, so preventing the animal from burying itself completely.
Fig. 11. To determine the relation between the magnitude of the response and the intensities of the light and contact stimuli.

A. The response measured at varying contact intensity for three constant light intensities $L_1$, $L_2$, and $L_3$.

B. Converse experiment to A. The response measured at varying light intensity for three values of contact intensity $C_1$, $C_2$, and $C_3$.

C. The relation between the intensities of the light and contact stimuli for three magnitudes of response $R_1$, $R_2$, and $R_3$. The relation can be obtained from either A or B.
Fig. 12. The relation between the proportion of "reactions" and the logarithm of the light intensity in the absence (curve Co, open circles) and presence (curve C1, full circles) of a contact stimulus. The graphs are plotted from the data given in Tables 12 and 13.
Fig. 13. The swimming movement of the ammocoete. Redrawn from alternate photographs in a 64 exposures per sec. film record. Vertical lines represent the same grid line, horizontal lines successive grid lines. The crests (small circles) of the contraction waves recede relative to the ground.
Fig. 14. The burying movements of the ammocoete. Redrawn from a 16 exposures per sec. film record of an animal burying in the water cell; A to Q represent successive exposures. Vertical lines represent the same grid line; horizontal lines mark the surface of the sand. A to G show the initial penetration of the sand, H to P the lateral head movement preceding the formation of a single curve (Q&R), which is later extended along a sinusoidal track (S to V).

Fig. 15. As in Fig. 14, but showing the formation of an initial curve (L to N) and its obliteration by later curves (O to T) as more of the body enters the sand. A to H initial penetration, I to K lateral head movement. A to M represent successive exposures.
Fig. 16. The burrowing movement of the ammocoete. Redrawn from every fourth photograph in a 16 exposures per sec. film record of an animal moving over the surface of wet sand. A and B represent respectively the same grid lines; horizontal lines represent successive grid lines. The crests (small circles) of the contraction waves remain approximately stationary relative to the ground,
Fig. 17. The single line track left by an ammocoete moving over the surface of wet sand.
Fig. 18. The lateral movement of the head in an animal moving over the surface of wet sand. Successive positions of the tip of the head are shown in relation to the track (dotted line) followed by the rest of the body. Movements of the oral lips transform the shape of the head into a wedge pointing in the direction of the head movement. Redrawn from a 16 exposures per sec. film record.
Fig. 19. The form of the body during the burrowing movement for

A. an animal on the surface of wet sand
B. an animal in the water cell.

An animal on the surface of wet sand exhibits a smaller number of curves than an animal in the water cell.
Fig. 20. The occurrence of the "short" and "long" reaction times and the swimming and burrowing movements in relation to varying contact. The variation in contact is expressed for the present purpose by the area of body against which contact is maintained.

a-c is a scale representing increasing area of contact.
a-b the swimming movement occurs: it does so with a "short" reaction time.
b-d the burrowing movement occurs: it does so with either a "short" or "long" reaction time.
Fig. 21. The decrease in the reaction time for ammocoetes of *L. fluviatilis* obtained from two different fertilizations (full circles and open circles) shown first in relation to the length of the animal and secondly in relation to time from hatching. The reaction times from the two populations approximate more closely when plotted in relation to length. The points represent single measurements of the reaction time over a light intensity range of 13.3–26.9 f.c.
Fig. 22. The decrease in the reaction time during the development of the light response for *L. planeri*. A maximum of five single measurements of the reaction time obtained at 1.5 and 2.5 f.c. (full circles) and 80.7 and 140.6 f.c. (open circles) are shown for each length class. The graph is plotted from data given in Table 16.
Fig. 23. The decrease in the reaction time during the development of the light response for L.fluviatilis. A maximum of five single measurements of the reaction time obtained at 0.2 and 0.4 f.c. (full circles) and 44.3 and 72.4 f.c. (open circles) are shown for each length class. The graph is plotted from data given in Table 17.
Fig. 24. The decrease in the reaction time during the development of the light response for two ammocoetes of *L. planeri*. Single measurements of the reaction time are plotted in relation to length for two light intensities. Full circles 2.5 f.c. in A, 13.1 f.c. in B. Open circles 80.7 f.c. in A, 140.6 f.c. in B. Dotted line indicates the occurrence of a "no reaction" between measurements. Full line shows a response was obtained at each successive test.
Fig. 25. The decrease in the reaction time during the development of the light response for two ammocoetes of L.fluviatilis. Single measurements of the reaction time are plotted in relation to length for three light intensities. Full circles 0.2 f.c. in A, 0.7 f.c. in B; half circles 2.7 f.c. in A, 7.2 f.c. in B; open circles 27.0 f.c. in A, 72.4 f.c. in B. Dotted line indicates the occurrence of a "no reaction" between measurements. Full line shows a response was obtained at each successive test.
Fig. 26. The development of the light response in relation to time for *L. planeri*. The approximate durations of the main changes in the reaction time (RT) and proportion of "no reactions" (NR) are marked on a growth curve for the animal. Each point on the growth curve is the mean of six measurements from a total of six animals. The time scale has an arbitrary origin.
Fig. 27. The development of the light response in relation to time for *L.fluviatilis*. The approximate durations of the main changes in the reaction time (RT) and the proportion of "no reactions" (NR) are marked on a growth curve for the animal. Each point on the growth curve represents the mean of four measurements from a total of four animals. The time scale has an arbitrary origin.
Fig. 28. To show the length of the ammocoete at which stages in the development of 1. swimming movement 2. movement in response to light and 3. burrowing behaviour occur. Each point represents the length of a single animal on which the observation was made. Arrows indicate the development has reached its final form. Hatching lengths are also shown. Figure constructed from data obtained from L.fluviatilis.
Fig. 29. The morphological development of *L. planeri*. The length of the animal and approximate time intervals from the commencement of observations are indicated for each stage of development. Animals were measured along an axis parallel to the main axis of the body. e, endostyle; gp, gill pouches; h, heart; l, liver; p, pit; s, stomodaeum. Full description is given in text. Camera lucida drawings.
Fig. 30. The main stages in the morphological development of *L. fluviatilis*. Animals measured along a line parallel to the main axis of the body. Camera lucida drawings.
ABSTRACT OF THESIS

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Title of Thesis: Studies on the Light Response of the Ammocoete Larva of Lampetra planeri (Lösch) and Lampetra fluviatilis (Linn.)

An investigation was made of a modification in the response to light of the ammocoete larva after the introduction of a thigmotactic stimulus. The response to light of the ammocoete is a random movement; between the application of a light stimulus and the resulting movement there is a delay or reaction time whose duration varies inversely with the intensity of light. When only a light stimulus was applied the reaction time varied from 1 to 3 sec. over a respective intensity range of 100 to 1 foot candles. The variability of a number of measurements of the reaction time at a given intensity was greater at lower intensities. The thigmotactic stimulus was applied by allowing the animal to lie in contact with sand. In the presence of a thigmotactic stimulus the reaction time was longer than that obtained at similar intensities in its absence; repeated measurements of the reaction time were more variable and on occasions the animal failed to respond. The increase in the reaction time occurred when the posterior part but not the anterior part of the body was in contact with sand. Long reaction times similar to those obtained in the presence of a thigmotactic stimulus were obtained in its absence at light intensities ranging from 0.001 to 0.1 f.c. A change in the long and variable reaction times was assessed by the proportion of reaction times below a fixed value. It was thus possible to demonstrate a relation between the reaction time and light intensity in the presence of a thigmotactic stimulus and to compare this relation with that obtained in the absence of a thigmotactic stimulus. There was an indication that the length of the reaction time depended on the size of the particle of which the sand was composed.

A description based on cine-film records is given of the movements whereby the ammocoete buries itself in sand. The locomotory movement occurring beneath the sand resembles the serpentine movement of the grass-snake; there are, however, movements of the head and lips which assist in displacing the sand.

At an early stage of development, shortly before hatching, the light response was absent. When the response first appeared the reaction times were long. Thereafter the reaction time gradually decreased over a period of 0.3 days until it became short and similar to that of older ammocoetes. Previous exposure to light was not required for the development of the short reaction time. The long reaction times that occurred in the presence of a thigmotactic stimulus in older ammocoetes were not obtained in ammocoetes in which the light response had newly developed.

The reaction time is partly the time required for the appearance of afferent impulses in the nerves leading from the photoreceptors and partly the time required for the afferent impulses to generate impulses in the motor nerves. The thigmotactic stimulus inhibits the activity of motor nerves and so increase the time required for their excitation.
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