THE MECHANISMS OF TROPISTIC REACTIONS AND THE STRYCHNINE EFFECT IN DAPHNIA

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I

Some knowledge of the nature of tropistic reactions has been gained through the investigation of their reversal by various agents (Ewald, 1912, 1914; von Frisch and Kupelwieser, 1913; Loeb, 1918; Crozier and Arey, 1918; Mainx, 1929; Welsh, 1930; Clarke, 1930, 1932). Clarke found for *Daphnia* that geotropism and phototropism could be temporarily reversed by abruptly changing the intensity of light falling upon the animal. A tentative explanation of the reactions of *Daphnia* to light was proposed by postulating the involvement of a reversible photochemical process in the photoreceptors (*cf.* Hecht, 1929, 1931). This interpretation of the responses of *Daphnia* to photic stimulation largely depends, however, upon two assumptions.

First, it was assumed that in *Daphnia* the mechanism for orientation is distinct from that underlying the other aspects of phototropism investigated (Clarke, 1932). Such an assumption was made by Ewald (1914) for the interpretation of his results obtained by stimulating *Daphnia* with intermittent and with colored light. Moreover, the assumption is strongly supported by the fact that changes in illumination which reverse the sign of phototropism do not also reverse the orientation of the body: the animal tends to maintain a position with its dorsum toward the light source under all circumstances (Fig. 1).

Secondly, it was assumed that a change of sign of phototropism involves the control of antagonistic muscles in the swimming appendages which are reciprocally innervated. It is conceivable that the postural angle of the antennae which are chiefly responsible for locomotion is controlled directly by the impulses arising in the photoreceptors. The result would be that, following a change in light intensity, the antennae would be forced by changes in muscular tensions into such a position that subsequent swimming movements would carry the animal toward the light. Observation, however, does not tend to support a strict interpretation of this view, if it involves the proposed mechanism alone. For, under these circumstances *Daphnia* would never move in a positive direction except when the tilted backwards position for swimming was assumed. It has been reported that *Daphnia* suddenly made photopositive *sometimes* turn around and swim frontwards, scuttle from side to side in a zig-zag

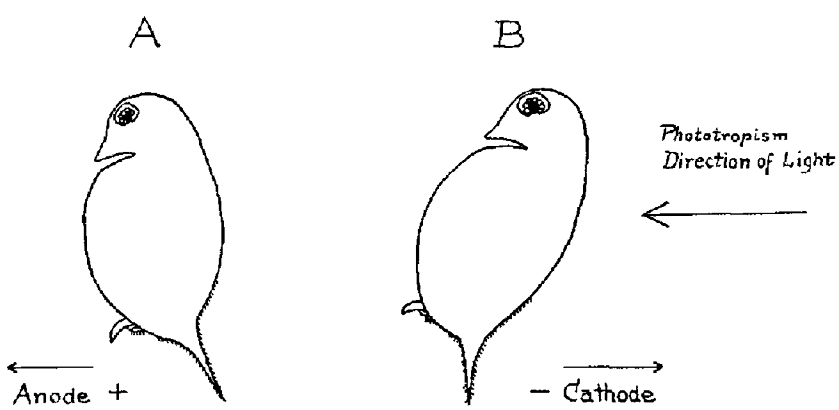

FIG. 1. Diagram to illustrate the usual position of *Daphnia* (*A*) when swimming "forward" and (*B*) when swimming "backward." (*A*) represents an individual which is negatively phototropic or anodically galvanotropic, (*B*) represents an individual which is positively phototropic or cathodically galvanotropic. Note that in both cases the organism "faces" in the same direction. Antennæ omitted.

course, or even bump along the bottom head down (Ewald, 1914; Clarke, 1932).

Accordingly, we are not in a position at present to decide whether stimuli from the photoreceptors produce directly a change in position of the antennae which automatically results in a characteristic phototropic movement, or whether the stimulus arouses a general tendency to move toward or away from the light which secondarily calls forth suitable swimming movements. However, we do know that *Daphnia* swims tilted forward when it is photonegative and usually swims tilted backward when it is photopositive. This difference in position could be accounted for by the presence of antagonistic muscles in the antennae controlled by reciprocal innervation. A stimulus from the photoreceptors evoking the forward position of the antennae would result in negative phototropism, and a stimulus producing the opposite position of the antennae would result in a tilting of the animal's body backward and a photopositive swimming movement.

Since the action of strychnine has been used as a test of the presence of reciprocal innervation in reflexes (*cf.* Knowlton and Moore, 1917; Moore, 1918–19, 1923–24; Crozier, 1919–20, 1927–28; Crozier and Federighi, 1924–25; Fries, 1927–28) it was proposed to use this method in studying the responses of *Daphnia* to light and to galvanic current. Modifications of tropistic reactions following strychninization should serve as a test for the assumptions under consideration.

II

To test the effect of strychnine upon the phototropism of *Daphnia*, single animals were placed in a glass trough (8 cm. long, 3.5 cm. wide, and 2.5 cm. high) illuminated from one end (*cf.* Clarke, 1932). After the usual phototropic reactions to light had been tested, the animal was removed from the trough and placed in a dilute solution of strychnine sulfate in pond water (1:200 of the saturated strychnine solution). After a few minutes the animal was transferred back to the trough and its responses again tested. This procedure was repeated until the animal became indifferent to the light stimulus.

During the repetitive tests *Daphnia magna*[1] which were originally negatively phototropic become constantly photopositive to all light intensities after 6 to 10 minutes in the strychnine solution. Animals which were originally primarily photopositive remain constantly positive. In both cases the usual responses to *changes* of illumination are abolished; neither an increase nor decrease of intensity of light produced any reaction. The orientation of the animal (its back toward the light) is not altered at any time. After a total stay of about 15 minutes in strychnine the animals begin to somersault, and soon fail to show any phototropic swimming movements. The occurrence of this condition probably accounts for the report that strychnine destroys phototropism in *Daphnia* (Moore, 1912). If the animals

[1] This and the other species tested were kindly furnished by Prof. A. M. Banta from his cultures.

are left longer in the strychnine, tetanic spasms follow and continue until the animals die after about 6 hours.

In the case of *Daphnia longispina* all animals tested were found to be primarily positively phototropic to all light intensities. Like *Daphnia magna* they became temporarily photonegative following sudden increase of light intensity. After 3 to 5 minutes in strychnine solution of the same concentration, the positive phototropism is destroyed. Although the animals tend to swim away from the light, the swimming movements are no longer normal, a good deal of somersaulting being exhibited. Rapid changes of light intensity now evoke no response. Longer sojourns in strychnine result in more frequent somersaulting and soon all phototropic movements are abolished.

III

The galvanotropism of *Daphnia* was investigated by placing the animals in a trough of pond water similar to the one described above and passing a galvanic current through the water by means of zinc electrodes embedded in cotton pads at each end. The observations had to be made in a dark room, to avoid any conflict between a galvanotropic and a phototropic response. The trough was illuminated by a weak light placed just above the trough. When subjected to current densities of 0.5 to 1.0 milliamperes per $cm.^2$, *Daphnia magna* exhibits a strong anodic galvanotropism. The reaction is very much more vigorous when the circuit is first closed. To a continuous current *Daphnia* becomes progressively less responsive until the current density is increased or the circuit is broken and made again. This is probably due to polarization within the animal. To weaker currents the organism is indifferent, whereas stronger currents produce general tetanic contraction which prevents the animal from swimming. Under these conditions the *Daphnia* sinks to the bottom, but it always lies on its back with the head toward the cathode. When the direction of the current is reversed, the animal turns itself around.

After 6 to 10 minutes in the strychnine solution *Daphnia* becomes strongly cathodic. The orientation to the direction of the current, however, remains the same. The body is tilted backward and the *Daphnia* progresses thus toward the cathode (Fig. 1). When subjected to stronger currents, the animal falls to the bottom lying on its back with its head to the cathode exactly as before. This condition with reversed sign of galvanotropism but with orientation unchanged persists until tetanic spasms begin after about 30 minutes. Strychninized animals generally die after 1 hour when repeatedly exposed to the current. This is very much faster than in the case of the experiments involving stimulation by light.

In *Daphnia longispina* the galvanotropic responses were found to be essentially the same as in *Daphnia magna*. *Moina macrocopa*, however, exhibits a strong cathodic response. A current of 2.0 to 2.5 milliamperes per $cm.^2$ is required in this case. Treatment with strychnine does not alter the sign of galvanotropism and even after 30 minutes in strychnine solution *Moina* is still cathodic. This form, however, is so small that the position of the body during orientation cannot be observed with the naked eye.

IV

These experiments show that strychnine has a pronounced effect upon the sign of phototropism and of galvanotropism, but the orientation of the animal is not affected in either case. Thus strong support is provided for the assumption that the mechanism for *orientation* is distinct from the mechanism responsible for the *sign of phototropism*.

Confirmatory evidence was also obtained for the second assumption, that the antennae are controlled by reciprocally innervated antagonistic muscles. When strychnine is successfully employed as a means of testing for reciprocal innervation, the altered response of the neuromuscular system to a given stimulus is usually regarded as due to a "reversal of inhibition" in the reflex arcs (Sherrington, 1906). There are, however, other possible explanations for different cases: the drug might affect directly the receptors concerned (*cf.* Crozier and Federighi, 1924–25), or produce a reversal of tropism without the occurrence of simultaneous contraction of antagonistic muscles (Crozier, 1920); or, finally, strychnine might increase irritability to such an extent that stimuli normally producing a simple reflex, produce tetanic contractions of the whole muscular system similar to that in the "start" reflex (Cushny, 1919).

Although these other interpretations of the strychnine effect have not been excluded, the "reversal of inhibition" theory seems to offer the most satisfactory explanation of the results with *Daphnia*. According to this view the effect of the drug is to change normal reflex inhibition into excitation, with the result that both sets of antagonistic muscles are stimulated to contract simultaneously. The stronger set of muscles is believed to overpower the weaker set. *Daphnia* treated with strychnine would respond to a stimulus by simultaneous contraction of both sets of muscles in the antennae. Since strychnine produces positive phototropism, the conclusion follows that the set of muscles producing the back or photopositive position of the antennae is stronger than the set producing the forward or photonegative position. A change in illumination no longer produces a change of the sign of phototropism because both sets of muscles are already in a state of increased tonus and the stronger continues to maintain the animal in the photopositive posture. Those specimens of *Daphnia* which are normally primarily photopositive continue to show the same phototropic response after strychninization. This condition can be understood on the same basis, for, if the back set of muscles is the stronger, they will control the sign of the reaction whether or not the forward set is also stimulated to contract.

The results of the experiments on galvanotropism may be interpreted in the same way. The orientation mechanism is not affected by strychnine and hence the *Daphnia* always faces in the same direction,—toward the anode. Normally the animal swims with the forward set of antennal muscles contracted and the backward set inhibited and relaxed. After a short stay in the strychnine solution, however, both sets of muscles are stimulated to contract, and, if the back muscles are the stronger, according to the deduction made above, the animal would be forced to swim tilted backward. The result is that *Daphnia* now moves to the cathode although it continues to face in the opposite direction.

SUMMARY

1. Experiments with strychnine were performed to test two assumptions important in the development of a theory for the mechanisms involved in the tropisms exhibited by *Daphnia*.

2. After a brief interval in strychnine solution *Daphnia* exhibits a reversal of the primary sign (*a*) of phototropism, from negative to

positive; and (*b*) of galvanotropism, from anodic to cathodic. In both cases the orientation of the body remains the same.

3. The mechanism responsible for the sign of phototropism and galvanotropism in *Daphnia* is therefore distinct from that underlying orientation.

4. Evidence is obtained indicating that changes in sign of tropism, produced by changes in illumination or by subjection to strychnine, involve the control of antagonistic muscles in the swimming appendages which are reciprocally innervated.

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