Determinants of planarian aggregation behavior

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Using a filter technique, five experiments examined the role of chemical and visual cues in recruiting individual planarians to established aggregations. The results indicated that *Cura foremani* depend upon a combination of chemical and visual cues while *Planaria dactyligera* depend almost entirely on chemical cues for aggregation formation.

For several years we have been investigating a primitive form of social behavior in planarians. Our research indicates that aggregation formation in planarians is a general effect observed in several planarian species (Reynierse & Ellis, 1967), probably occurs as a response to both chemical and visual stimuli (Reynierse, 1966; Reynierse, Gleason, & Ottemann, 1969; Reynierse & Scavio, 1968), and is especially relevant for planarian reproductive activity (Reynierse, 1967; Reynierse, Auld, & Scavio, 1969). Presumably, aggregated planarians secrete a pheromone substance to which other planarians are positively chemotactic, thus accounting for the addition of new individuals to established aggregations (Reynierse, Gleason, & Ottemann, 1969) and the formation of large aggregations both naturally and in the laboratory. Similarly, aggregations of planarians contrast with lighter backgrounds, providing a visual stimulus for attracting new planarians (Reynierse & Scavio, 1968).

Although our earlier research provided indirect evidence for a pheromone interpretation, recourse to the pheromone concept requires that the implicated chemical cue be secreted to the external environment where it is received by a conspecific individual, producing a complete behavior response in that conspecific (Gleason & Reynierse, 1969). Thus, free-moving planarians, having detected the pheromone, should make directed movements toward an existing aggregation. Using filters which are sufficiently large to accommodate pheromones but which differentially permit visual cues to operate, five experiments examined further the role of olfactory and visual cues in planarian aggregation behavior.

METHODS

From laboratory populations of *Cura foremani*, a total of 390 planarians used in Experiments 1 and 2 and an additional 58 used in Experiment 3 were randomly selected without replacement. From laboratory populations of *Planaria dactyligera*, about 100 planarians used in Experiments 4 and 5 were randomly selected without replacement. Usual maintenance conditions, described elsewhere in detail (Reynierse, 1966, 1967; Reynierse, Gleason, & Ottemann, 1969; Reynierse & Scavio, 1968) were observed. With the exception of Experiment 5, which was conducted in the dark, experimental sessions were conducted under a diffuse light source, with room temperature $72^{\circ}\text{F} \pm 2^{\circ}\text{F}$. The test apparatus was thoroughly cleaned, rinsed with aged tap water, and airdried between experimental sessions.

The test apparatus, shown in Figure 1, consisted of two identical lucite chambers with overall internal dimensions of $7\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{1}{2}$ in. Each chamber could be divided into three identical $2\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{1}{2}$ in. compartments when filters were fitted between the middle and end sections. Equal amounts of aged tap water were added to a depth of approximately 1 in. to each compartment. A grid divided into 10 equal sections was under and clearly visible through the middle compartment, permitting giving a location score to planarians in this section.

Figure 1. Test chamber with dialysis tubing filter fitted between the middle and end compartments.

Figure 2. Mean location scores for three (aggregation, conditioned, or plain) end compartment cue source conditions.

EXPERIMENT 1

If planarians are directed to aggregations by a pheromone originating from the aggregation, free-moving individuals should move toward the cue source and come to rest nearest that source if prevented from actually joining the aggregation. This experiment compared the responses of free-moving individual planarians in the middle compartment under three combinations of end compartment cue sources.

Procedure. For all groups, a single thickness of Type C dialysis tubing, cut into a single sheet, was fitted between each end and middle compartment section, permitting molecules of the size generally attributed to pheromones to pass easily from an end compartment to the middle compartment. In the aggregation group, 15 *C. foremani* were introduced into an end compartment where they formed aggregations during an 18-h period; the other end compartment contained only aged tap water. In the conditioned group, 15 *C. foremani* were also introduced into an end compartment where they formed aggregations. But, after 18 h, these planarians were carefully removed with a micropipette, leaving only the conditioned water. The other end section again contained aged tap water. In the plain group, planarians were not added to either end compartment and both end sections contained only aged tap water. Four observation periods completed a 3 by 4 factorial design with observation periods as a repeated measure. Six replications of each experimental condition were run randomly.

Adding a single *C. foremani* to the center of the middle compartment and recording its position relative to the underlying grid at the end of 15, 30, 45, and 60 min constituted a test session for all groups. Location scores were assigned from "1," nearest the end compartment containing aggregations or conditioned water, through "10," nearest the end containing plain water. For the plain group the grid section designated as "1" was randomly alternated.

Results

Mean location scores for each group are summarized in Figure 2. Irrespective of experimental condition, every planarian came to rest during the 60-min observation period, but their location scores were a

function of cue source. A repeated measures analysis of variance yielded only a significant effect for cue source ($F = 11.95$, $df = 2/15$, $p < .01$). Using Duncan's multiple range test, the location score for the aggregation group differed significantly from both the conditioned and plain groups ($p < .001$) while the conditioned and plain groups were not significantly different from each other. Every planarian in the aggregation condition came to rest as near as possible to the existing aggregation in the adjacent compartment; in contrast, animals in the conditioned and plain groups were distributed randomly throughout the compartment.

EXPERIMENT 2

Pheromones are generally known to have molecular weights between 80 and 300 (Wilson & Bossert, 1963). Since dialysis tubing will accommodate molecular weights to a magnitude of approximately 12,000, it clearly permits chemical cues to operate. However, dialysis tubing is also transparent and clearly permits visual cues of an aggregation to operate as well. This experiment compared the responses of free-moving *C. foremani* in the middle compartment to the cues originating from aggregations in an end compartment and filtered by either Type C dialysis tubing (accommodating molecular weights to a magnitude of approximately 12,000) or a Millipore filter (pore size = .01 microns and accommodating molecular weights to a magnitude of approximately 50,000). The two filters both permit filtering chemical cues, while visual cues should operate only for the transparent dialysis tubing and not for the opaque Millipore filter.

Procedure. The apparatus was fitted with either the dialysis tubing or Millipore filters and aged tap water was added to each section. Fifteen *C. foremani* were introduced into an end compartment where they formed aggregations during an 18-h period; as in Experiment 1, the other end compartment contained only aged tap water. A single free-moving *C. foremani* was introduced to the middle compartment and its location relative to the underlying grid was observed at the end of 15, 30, 45, and 60 min.

Four observation periods completed a 2 by 4 factorial design with observation periods as a repeated measure. Six replications of each experimental condition were run randomly.

Results

Mean location scores for each group are summarized in Figure 3. Again, every planarian stopped moving and came to rest before the 60-min observation period. The results of this experiment are unequivocal: individual planarians were located significantly closer to the cue source when it was filtered by dialysis tubing than by the Millipore filter ($t = 3.69$, $df = 10$, $p < .01$). A repeated-measures analysis of variance yielded a significant result only for this filter variable ($F = 6.05$, $df = 1/10$, $p < .05$). Finally, the results of the aggregation group in Experiment 1 were effectively

replicated, since every planarian without exception came to rest in the grid section nearest the aggregation in both experiments when dialysis tubing was the filter.

EXPERIMENT 3

Considered together, the results of Experiments 1 and 2 favor the role of visual stimulation as the primary determinant of planarian aggregations in *C. foremani*. However, the two experiments only permit evaluating the role of olfaction alone (Millipore filter) or the joint effects of olfaction and vision (dialysis tubing). It is clearly desirable to design a test in which the visual component is separated from olfactory stimulation. In this connection, Reynierse and Scavio (1968) and Reynierse, Gleason, and Ottemann (1969) report that *C. foremani* aggregate on contrasting tape, although, again, the joint effects of vision and olfaction cannot be eliminated. Nevertheless, if individual planarians would reliably come to rest on black tape, this effect would provide convincing evidence for the superiority of visual stimulation in accounting for planarian aggregation behavior.

Procedure. The apparatus was fitted with either dialysis tubing or Millipore filters and aged tap water added to each section. A 30 x 18 mm piece of black tape was attached to one side of one of the strips of dialysis tubing and one of the Millipore filters. In each case the tape was on the surface of the filter facing the end compartment while the other end compartment contained only the appropriate filter. A single free-moving *C. foremani* was introduced to the middle compartment and its location relative to the underlying grid was observed after 60 min. A total of 29 *C. foremani* were run for each of the two filter conditions.

Results

Mean location scores after 60 min were 5.82 under the Millipore filter condition and 4.22 under the dialysis tubing condition, a difference which was not significant ($t = 1.3$). In contrast to Experiments 1 and 2, 7 planarians in the Millipore filter condition and 2 planarians in the dialysis tubing condition were still active after 60 min and were not included in the statistical analysis. These results are clear: within the limits of this experimental test, contrasting black stimulation by itself cannot account for planarian aggregation formation.

EXPERIMENT 4

This experiment examined aggregation formation in *Planaria dactyligera*, a planarian species which forms unusually compact aggregations (Reynierse & Ellis, 1967) and which may be particularly dependent upon olfactory cues for its aggregation behavior.

Procedure. The apparatus was fitted with either dialysis tubing or Millipore filters and 25-40 *P. dactyligera* were introduced into an end compartment where they formed aggregations. After

aggregations were established, a single free-moving *P. dactyligera* was introduced to the middle compartment and its location relative to the underlying grid was observed after 60 min. A total of 10 planarians were run under each of the filter conditions.

Results

In every case, one or two very compact aggregations were formed directly on the filter surface in the end compartment. Under the dialysis tubing condition, 9 out of 10 planarians obtained a location score of "1" (mean = 1.9), coming to rest as near as possible to the aggregation. Under the Millipore filter condition, 7 out of 10 planarians obtained a score of "1" (mean = 2.7). Most importantly, regardless of filter, whenever a score of "1" occurred, the single planarian in the middle compartment always came to rest on the filter immediately opposite to an established aggregation. This is particularly remarkable since each aggregation covered only a small fraction of the total surface of any filter. These results suggest that aggregation formation in *P. dactyligera* is mediated primarily by olfactory pheromones and probably not by vision, although the role of visual cues cannot be eliminated.

EXPERIMENT 5

This experiment is a particularly conservative test of the pheromone hypothesis, examining the aggregation behavior of *P. dactyligera* in the dark.

Procedure. This experiment replicates the procedures of Experiment 4, except that it occurred in an unlighted room painted flat black. Aggregations were noted and scores recorded under dim illumination at the end of each experimental session. A total of 8 planarians were run under each of the filter conditions.

Figure 3. Mean location scores obtained with dialysis tubing and Millipore filters.

Results

As in Experiment 4, in every case, compact aggregations formed directly on the filter surfaces. However, individual *P. dactyligera* did not reliably collect near established aggregations. Under dialysis tubing, 6 out of 8 planarians obtained a location score of "1" (mean = 3.0) while under the Millipore filter, 4 out of 8 planarians obtained a location score of "1" (mean = 4.4). Furthermore, two of these scores in each of the filter conditions were away from rather than directly opposite established aggregations. These results cannot be interpreted unambiguously and suggest that some factor other than a pheromone was also influencing the behavior of *P. dactyligera*. Nevertheless, that aggregations always formed in darkness where visual cues were inoperative as well as the fact that on six occasions a single planarian in the middle compartment became inactive directly opposite an established aggregation, suggests that pheromones are a dominant determinant of aggregation formation in this species.

DISCUSSION

These results seem to be clear enough: *C. foremani* aggregate in response to the combination of chemical and visual cues while *P. dactyligera* seem to aggregate to chemical cues alone. There may be a danger in overinterpreting the results of Experiment 3, in which individual planarians did not systematically come to rest on or near the black tape visual cue. Nevertheless, within the contexts of Experiments 1 and 2, these results suggest that *C. foremani* do not aggregate to visual stimulation alone but rather depend on both chemical and visual cues. The primary role of pheromones for *P. dactyligera* is not challenged by the fact that several

individuals did not collect on or near an established aggregation under dark conditions. Rather, *P. dactyligera* appears to be a particularly inactive species (Reynierse & Ellis, 1967) and darkness may facilitate photonegative species to become inactive, regardless of their location.

The use of filter techniques in this set of experiments provides the most compelling evidence we have yet reported for the role of a pheromone in planarian aggregation behavior. Still, our experiments only provide indirect evidence for planarian pheromones, a limitation which will undoubtedly remain until pheromones are chemically isolated or can be shown to be differentially filterable.

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