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SENSORY BEHAVIOUR IN *DICTYOSTELIUM DISCOIDEUM* SLUGS: PHOTOTAXIS AND THERMOTAXIS ARE NOT MEDIATED BY A CHANGE IN SLUG SPEED

ELIZABETH SMITH[1], PAUL R. FISHER[2], WARWICK N. GRANT[2] AND KEITH L. WILLIAMS[1]*

[1] *Biochemistry Department, Faculty of Science, Australian National University, P.O. Box 4, Canberra City, A.C.T. Australia 2601*
[2] *Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, Federal Republic of Germany*

SUMMARY

The speed of sustained migration of *Dictyostelium discoideum* slugs was similar in a temperature gradient and at different light intensities, including a light intensity sufficient to cause significant disorientation of slugs. No change was observed in slug speed in the presence of high levels of Slug Turning Factor (STF), a low molecular weight compound through which phototaxis and thermotaxis are mediated. Thus orientation of *D. discoideum* slugs is not mediated by a sustained changed in slug speed and we propose that slug movement is not directly coupled to tactic responses. Slug speed depended on the size, age and genotype of slugs as well as the nature of the substratum (charcoal-containing water agar *versus* water agar).

INTRODUCTION

The slug stage of the cellular slime mould *Dictyostelium discoideum* is a simple multicellular organism, which exhibits a behavioural response (oriented migration) along gradients of light, heat and a chemical compound (Bonner, Clarke, Neely & Slifkin, 1950; Fisher, Smith & Williams, 1981). As such it provides a system for the study of behaviour at a level of complexity greater than that of the single cell (e.g. bacterial chemotaxis, Koshland, 1979) but without the added complexities of a highly developed nervous system (e.g. nematodes and insects, Hazelbauer, 1978). The existence of two easily studied taxes, to light and heat, offers some of the variety afforded in bacterial systems by chemotaxis to a range of compounds (Koshland, 1979).

Some features of sensory transduction involved in phototaxis and thermotaxis in *D. discoideum* are already understood. A striking finding is the wide response range in these types of behaviour, due presumably to adaptation mechanisms for sensing changes in the relative rather than absolute light and heat intensities (Bonner *et al.* 1950; Whitaker & Poff, 1980; Fisher *et al.* 1981).

* Author for correspondence.

In view of this adaptability, one surprising claim concerning slug phototaxis is the report (Poff & Loomis, 1973) that the migration rate of slugs is not adaptive but is proportional to light (signal) intensity. This point is controversial, since earlier reports (Bonner & Whitfield, 1965; Raper, 1940) failed to demonstrate an effect of light on slug speed. We have reinvestigated this question and show here that slugs migrate at the same speed in the dark (i.e. no signal), at light intensities sufficient to cause disorientation (i.e. saturating signal strength), and during thermotaxis in the dark. Slug speed is, however, dependent on slug size (Inouye & Takeuchi, 1979), age (Bonner, Koontz & Paton, 1953), genotype and the nature of the substratum.

MATERIALS AND METHODS

Preparation of young and aged slugs of D. discoideum

Slugs of *D. discoideum* strain NP84 (North & Williams, 1978) or NP187 (Mullens & Newell, 1978) were prepared as described previously (Smith & Williams, 1981). At appropriate times after the commencement of migration, 5-10 individual slugs were transferred to each of several fresh 9-cm diameter water agar (1·5% Difco agar + 250 μg/ml dihydrostreptomycin sulphate, 35 ml/plate), water agar ± 5 g/l activated charcoal (Ajax Chemicals, Sydney, N.S.W. Australia), or water agar ± Slug Turning Factor (STF) (Fisher *et al.* 1981) plates. All growth of amoebae and migration of slugs was conducted at 21 ± 1·0 deg. C (Smith & Williams, 1980).

Conditions for illumination and thermotaxis

Slugs migrated in the dark (Petri dish in an opaque PVC container) or towards the light (Petri dish inside an opaque PVC container with a hole 3 mm in diameter on one side). The light source was either a room lit with 40 W daylight fluorescent bulbs or a cold light pipe fitted 1 cm from a Bell & Howell projector with a Philips 24 V, 250 W, no. 6958 lamp. Light intensity measurements were made with an IL700 Research Radiometer (International Light) in such a way that the intensity experienced by slugs inside the dishes at the commencement of migration was measured. No infrared filters were used. For experiments on thermotaxis, Petri dishes in opaque PVC containers were placed on an aluminium heat bar in a temperature gradient of 0·2 deg. C/cm, with the midpoint of the plate being at 21·9 ± 0·2 deg. C.

Estimation of slug size and speed

One hour after transferring slugs, the size of each slug was determined from a drawing made with a camera lucida attached to a dissecting microscope. In most experiments relative rather than absolute areas were measured. However, in some experiments the absolute area was determined. The time taken for slugs to resume normal migration after transfer to fresh agar plates was found to be minimal. Speed was estimated by marking the position of transferred slugs either immediately or 24 h after transfer and again at about 24 h or 36 h (i.e. speed was estimated over a 24-h or 12-h interval). Sometimes the size of the slugs was measured again at 36 h. At the end of each experiment, permanent records were made by placing a clear PVC disc on the surface of each plate so that the slime trails adhered to it. The discs were then stained with Coomassie brilliant blue (Fisher *et al.* 1981). The distance travelled was estimated from electronically measured X, Y co-ordinates using a MOP digitizer tablet (Kontron, West Germany) by tracing the length of slime trail formed in a known time. Lengths and areas of slugs were also estimated using the MOP digitizer, with length being taken as the half-perimeter of the slug.

Preparation of STF

Two STF preparations (< 500 M_r exudate from developing amoebae) were made from strains XM1 and NP187 as has been described elsewhere (Fisher *et al.* 1981). Approximately 1000 phototaxis interference units (PIU) were spread on each 35 ml water agar plate (Fisher *et al.* 1981).

Estimation of the accuracy of orientation by slugs

The method of Fisher *et al.* (1981) was used.

RESULTS

Slug speed during prolonged migration

In this report we examine the effects of various treatments on prolonged migration, rather than short-term, maximum speed (Inouye & Takeuchi, 1979). Thus the values reported here represent the net effect of a combination of sustained movement and pulsatile movement (Inouye & Takeuchi, 1979; Smith & Williams, unpublished). It was obvious that slugs of different size differ markedly in speed (Bonner *et al.* 1953). To allow for the effect of size, slug speed was plotted against area (Fig. 1A) and in one case against length (Fig. 1B). Both measurements gave good regressions, although area was found to give a better fit to the data in this work for two reasons. Firstly, the area was more constant than the length when several measurements were made on the same slug at short time intervals; i.e. there was more variation in slug length due to the pulsatile nature of slug movement. Secondly, when old slugs and mutants with trailing rear ends were examined (Smith & Williams, 1981), it was often difficult to determine where the slug finished and the trail of cells commenced. This error was minimized by measuring areas, as the trail was quite a small percentage of the total slug area but it was a large proportion of its length. Fig. 1A, B shows the same two groups of NP84 slugs (young and old) with speed plotted against area (A) and length (B). The young slugs moved faster than the old slugs, although the slopes of the regressions from young and old slugs were similar (i.e. there was a constant change in the speed of slugs with age, independent of size). Fig. 1A shows that old slugs tend to be smaller than young slugs, presumably due to loss of cells from the tails of old slugs. Conversely, although old slugs are smaller, they tend to be longer than young slugs (Fig. 1B), because the rear of the slug becomes trailing with age (Smith & Williams, 1981). A decrease in speed with age was confirmed directly by determining the change in speed of a group of slugs over a long period (data not shown).

Speed of slugs migrating in the light or the dark

To determine the effect of light at the intensity used in our 'standard' phototaxis experiments, matched plates were prepared, one group for incubation in the dark and the other in the light (intensity $\sim$ 0·1 μW/cm^2). Fig. 2A shows that young slugs of NP84 incubated on water agar in the light had the same regression as slugs incubated on water agar in the dark. Fig. 2B, C shows experiments where young

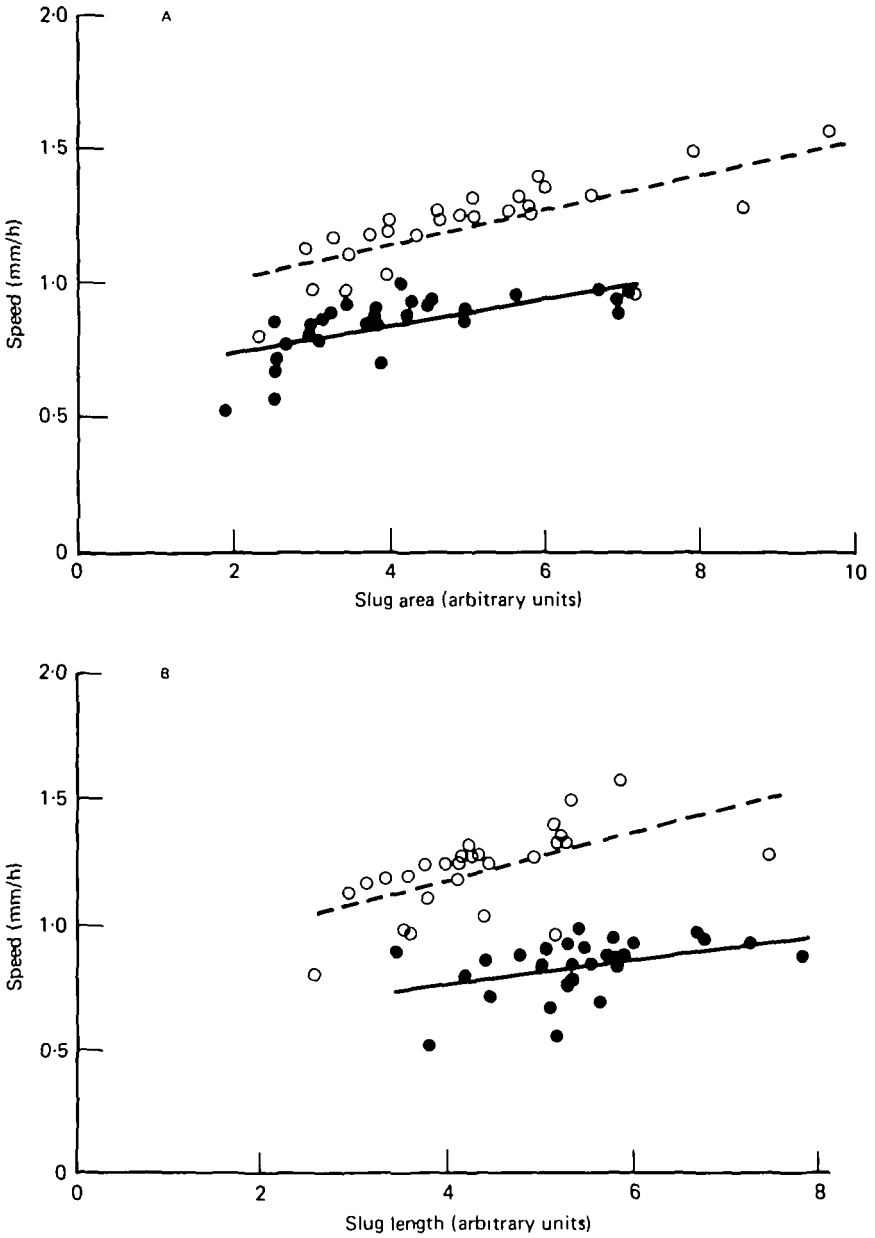

Fig. 1. Speed of young (o; less than 5-h migration prior to transfer) and old (●; 80-h migration prior to transfer) NP84 slugs migrated on water agar towards a light source ($\sim$ 0·1 μW/cm^2) plotted against (A) slug area and (B) slug length. A and B represent the same group of slugs and each point represents an individual slug. Linear regression lines are included for each set of results (---, young slugs; ——, old slugs).

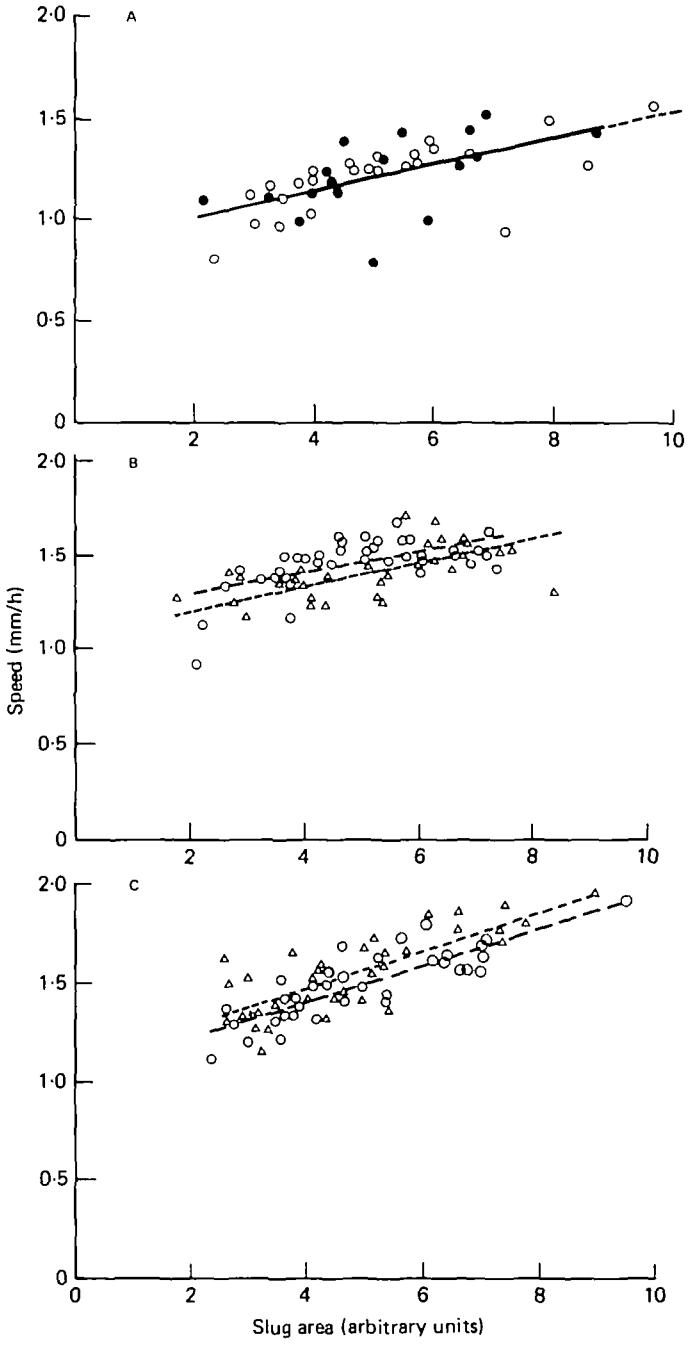

Fig. 2. Speed of young NP84 slugs migrating: A in the dark (●) and towards the light (○) (~ 0·1 μW/cm^2) on water agar; or towards a normal light source (○, ~ 0·1 μW/cm^2) and a bright light source (△, > 20 μW/cm^2) on water agar (B) or water agar +0·5 % (w/v) charcoal (C). Linear regression lines are included (——, dark; – –, light; - - -, bright light). Each point records the speed of a single slug.

NP84 slugs were incubated on water agar (Fig. 2B) or charcoal agar (Fig. 2C) with a normal light source ($\sim$ 0·1 μW/cm^2) and using a cold light source of much higher intensity ($>$ 20 μW/cm^2 experienced by the slugs). Neither on water agar (Fig. 2B) nor charcoal agar (Fig. 2C) was the regression for slugs illuminated at high light intensity much different from that of the normally illuminated slugs. Therefore, slug speed was independent of light intensity.

Disorientation of slugs migrating towards a bright light source

It was noted that slugs migrated with poor orientation at high light intensity (Fig. 3) and that orientation was less accurate on water agar (Fig. 3C) than on charcoal agar (Fig. 3D). It has been established that phototaxis is impaired on water agar compared to charcoal-containing agar, due to an increase in light-scattering on water agar (Fisher & Williams, unpublished).

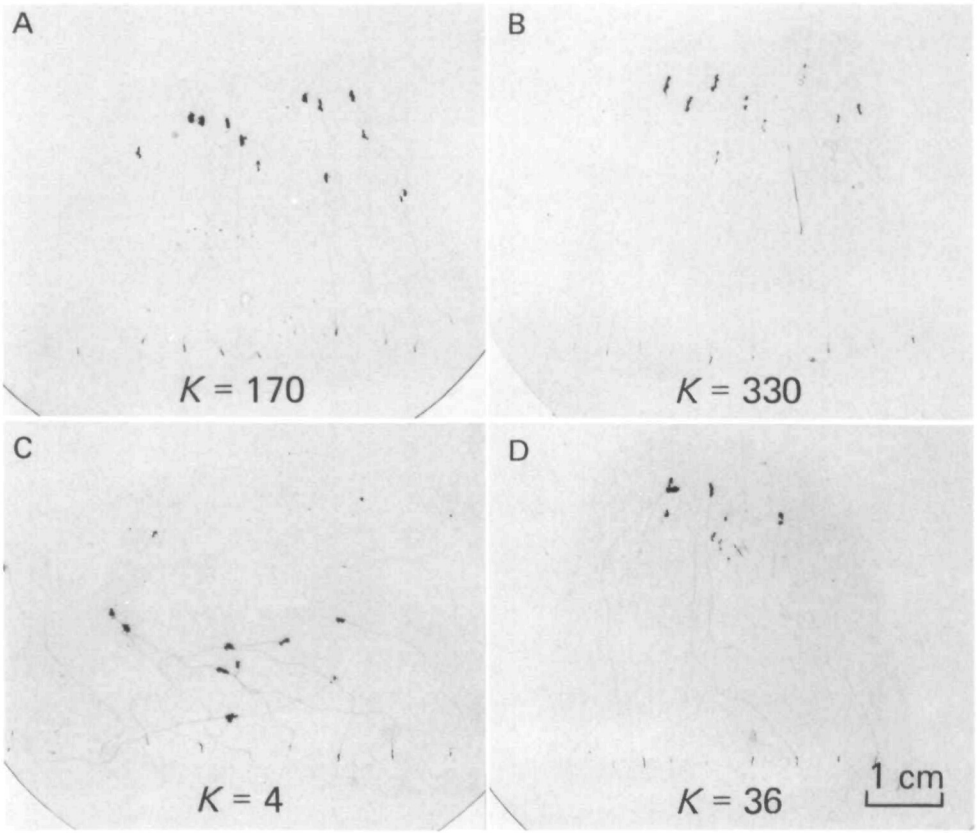

Fig. 3. Permanent records of trails of groups of 10 NP84 slugs migrating towards a light source (top of photograph) at a normal intensity of $\sim$ 0·1 μW/cm^2 (A, B) and high intensity of $>$ 20 μW/cm^2 (C, D). A and C migrated on water agar, while B and D migrated on water agar containing 0·5 % (w/v) charcoal. The migration of 40 slugs for each treatment was used to estimate the accuracy of orientation (K). K values given in the figure were calculated using circular normal statistics (Fisher *et al.* 1981).

Slug speed in the presence of STF

It has been shown (Fisher *et al.* 1981) that phototaxis in *D. discoideum* slugs is mediated by a light-stimulated low molecular weight repellent (STF). STF might conceivably act by increasing the speed of amoebae on the side of the slug distal to the light source. In two experiments we tested whether STF caused a sustained increase in slug speed by examining slug phototaxis in the presence of levels of STF sufficiently high to cause disorientation of NP84 slugs at normal light intensity. As is shown in Fig. 4, the speed of slugs migrating phototactically in the presence of high levels of STF was no different from that of control slugs migrating on water agar.

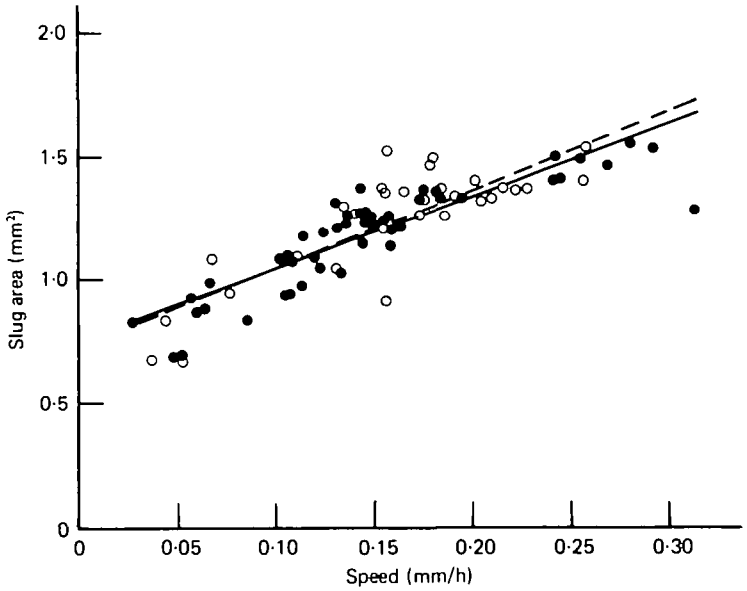

Fig. 4. Speed of young NP84 slugs migrating at a light intensity of ~ 0·1 μW/cm^2 on water agar (●) or water agar containing 1000 PIU of STF (○) (see Materials and Methods). Each point is the speed of an individual slug. Linear regression lines are included for both sets of results (——, water agar; – – –, water agar + STF).

Slug speed during thermotaxis

Slugs of *D. discoideum* are thermotactic, with the direction of thermotaxis determined by the growth and migration temperatures (Whitaker & Poff, 1980; Fisher & Williams, unpublished). Fig. 5 shows that NP84 slugs migrated in the dark at 21 °C in the presence of a temperature gradient of 0·2 deg. C/cm have the same speed as slugs migrated in the dark at 21 °C in the absence of a temperature gradient. Under the thermotactic conditions used, NP84 is positively thermotactic (accuracy of orientation, $K \approx 5$). Similar results were obtained for strain NP187 (data not shown).

Factors affecting slug speed

In the course of these experiments we confirmed that slug speed is size and age-dependent. Here we describe two other factors that influence slug speed.

Effect of charcoal on slug speed. It appeared from the experiments shown in Fig. 2 that slugs moved faster on charcoal agar than on water agar, so we tested this in an experiment where NP84 slugs migrated on water agar or charcoal–water agar under otherwise identical conditions. Fig. 6A shows that slugs do migrate ~ 30% faster on charcoal–water agar compared to water agar.

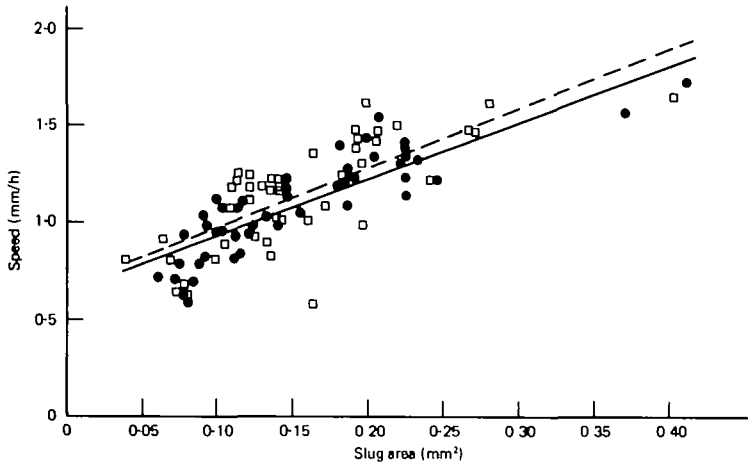

Fig. 5. Speed of young NP84 slugs migrating in the dark at 21 °C in the presence (□) or absence (●) of a temperature gradient of 0·2 deg. C/cm. Linear regression lines are included (——, no temperature gradient; – – –, temperature gradient).

Effect of genotype on slug speed. Fig. 6B shows that slugs of two genetically different strains of *D. discoideum* migrate at different speeds, NP187 being significantly slower than NP84. The slopes of the regressions are the same, however, indicating that the proportionality between speed and area is not altered.

DISCUSSION

Our finding that light does not increase the sustained speed of migration of *D. discoideum* slugs confirms and extends the earlier reports of Raper (1940) and Bonner & Whitfield (1965), but does not substantiate the contrary suggestion that slug speed increases in the light (Poff & Loomis, 1973). While the report by Raper (1940) is not detailed, that of Bonner & Whitfield (1965) gave convincing evidence using a different approach from that used here. No difference in slug speed was found when the speed of migration of individual slugs was examined after migration in alternating periods of light and dark (Bonner & Whitfield, 1965). We examined slug migration over a wide range of light intensities and found that at the highest intensity used there was significant disorientation of the slugs of strain NP84 used in this work (Fig. 3). This is the first report of such disorientation by high light intensity. From this we conclude that the extremes in effective range of light intensities have been examined here without a significant change in slug speed. We confirmed previous reports (Bonner *et al.* 1953; Inouye & Takeuchi, 1979) that

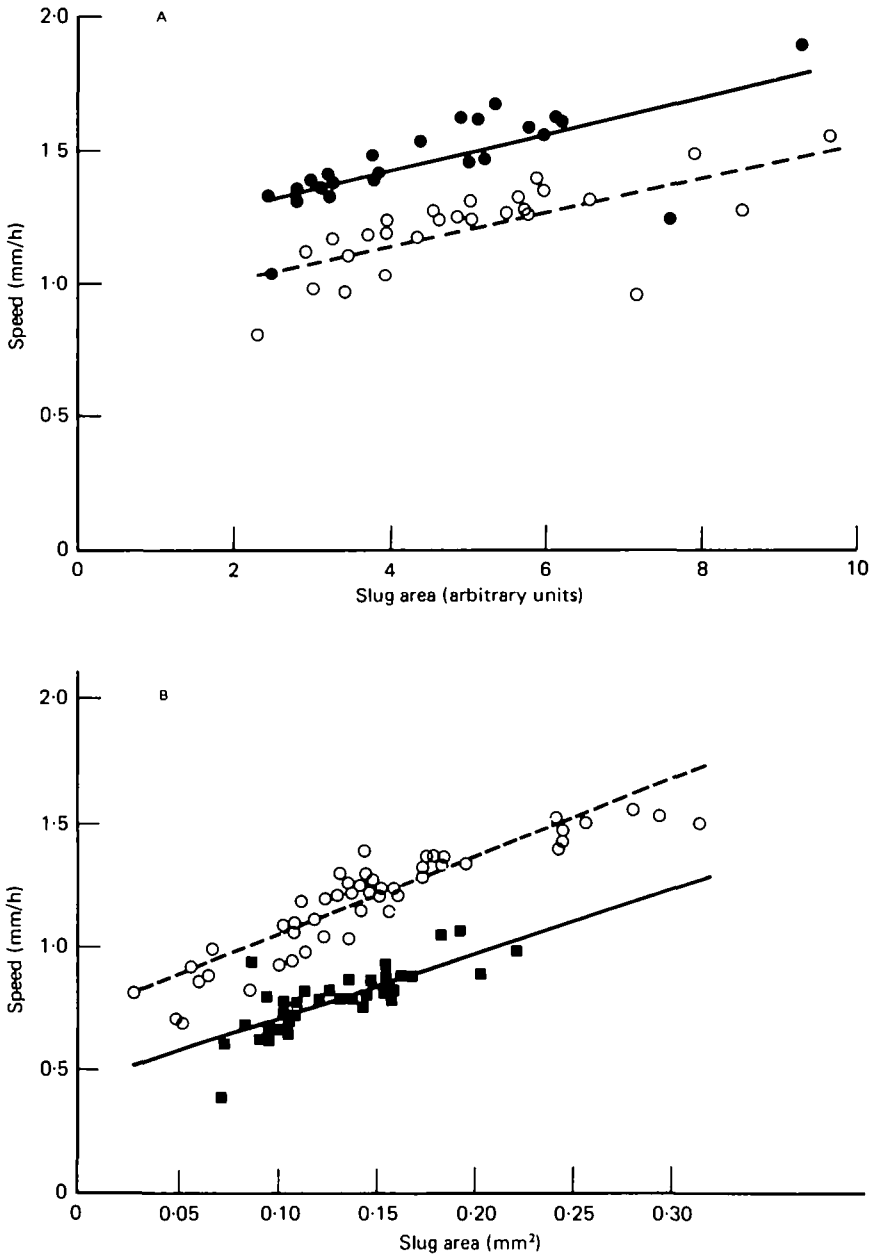

Fig. 6. Factors affecting slug speed. A. Speed of young NP84 slugs migrating at a light intensity of 0·1 μW/cm^2 on water agar (○) or 0·5 % (w/v) charcoal–water agar (●) under otherwise identical conditions. Each point is the speed of an individual slug. Linear regression lines are included for both sets of results (——, charcoal–water agar; – – –, water agar). B. Speed of young slugs of strains NP84 (○) and NP187 (■) prepared and migrating under identical conditions in normal light on water agar. Regression lines are included (– – –, NP84; ——, NP187).

speed varies markedly with slug size, and since Poff & Loomis (1973) did not measure the size of their slugs, this could explain their different findings.

In this study the speed of migration has been examined over a prolonged period. It is possible that a change in direction leading to a turn towards a point light source is mediated by a transient increase in the speed of the cells at the tip of the slug distal to the light source (Francis, 1964; Poff & Loomis, 1973). Slug orientation involves a lens effect causing light to be focused on the side distal to the light source (Francis, 1964; Poff & Loomis, 1973), and we showed recently that light stimulates the production by slugs of a low M_r compound (STF) that repels them (Fisher *et al.* 1981). It is possible that light focusing causes a local increase in STF levels on the distal side of the slug, resulting in it turning, either due to an increase in speed of cells on that side of the slug or due to chemotaxis of these cells away from a localized STF build-up. Although slug movement is pulsatile (Inouye & Takeuchi, 1979; Smith & Williams, unpublished), in preliminary experiments using time-lapse filming we have been unable to detect any transient change in slug speed in response to changes in light intensity. Consequently, it seems more likely that orientation in light and heat gradients results from chemotaxis away from STF rather than a change in the speed of movement of cells in the slug.

The results presented here suggest that migration rate and taxis (or oriented movement) may not be closely linked, or at least that speed is not dependent on the nature of the signal. Slugs migrate in the dark (in the absence of any tactic stimulus) at the same speed as slugs migrate towards a light source (Fig. 2) or along a thermal gradient (Fig. 5). Therefore, movement in *D. discoideum* slugs can be separated into independent orientation and migration components, with orientation being imposed on the slugs' innate tendency to move at a constant average speed, regardless of the presence or nature of external stimuli. This notion is consistent with our observations using transplant experiments (Grant, unpublished) that orientation and speed are controlled by different cells within the slug. Orientation is a property of the tip, while speed is a property of the bulk of cells in the slug.

As well as substantiating previous reports that speed increases with slug size and decreases with slug age (Bonner *et al.* 1953; Inouye & Takeuchi, 1979), slug speed is shown here to depend on the genotype of the strain used and the substratum on which the slug migrates. We have observed that slugs migrating on charcoal agar move $\sim$ 30% faster than normal. A *D. discoideum* slug is a cohesive group of cells moving through a tube of slime sheath, which is continuously synthesized at the front and left behind as a collapsed tube at the rear to form the slime trail. The cells of the slug contact the slime sheath (which remains stationary on the substratum) rather than the substratum itself, so any effect of the substratum on slug speed must occur via modification of the interaction between slime sheath and slug cells. Modification of the slime sheath itself is the most probable means by which the substratum affects slug speed. Indeed, slugs migrating on charcoal agar have a less rigid slime sheath than identical slugs migrating on water agar. Hence it is possible that the normally rigid slime sheath may be the primary limiting factor in slug speed.

The most surprising finding about slug speed is, however, that all the regressions obtained for slug speed *versus* size have similar slopes, indicating that the relationship between slug area and speed is conserved and perhaps reflects a fundamental characteristic of slug migration. Recently, we have discovered an exception to this generalization in studies on slugs of diploid strains isogenic to the haploid strains used in this work. The only difference between such paired strains is ploidy-associated; for example, the ratio of cell surface area to cell volume is altered in diploids compared to haploids (see Stenhouse & Williams, 1981). These diploid strains may reveal new aspects of slug migration.

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REFERENCES

Bonner, J. T., Clarke, W. W., Neely, C. L. & Slifkin, M. R. (1950). The orientation to light and the extremely sensitive orientation to temperature gradients in the slime mold *Dictyostelium discoideum*. *J. cell. comp. Physiol.* **36**, 149–158.

Bonner, J. T., Koontz, P. G. & Paton, D. (1953). Size in relation to the rate of migration in the slime mold *Dictyostelium discoideum*. *Mycologia* **45**, 235–240.

Bonner, J. T. & Whitfield, F. E. (1965). The relation of sorocarp size to phototaxis in the cellular slime mold *Dictyostelium purpureum*. *Biol. Bull. mar. biol. Lab., Woods Hole* **128**, 51–57.

Fisher, P. R., Smith, E. & Williams, K. L. (1981). An extracellular chemical signal controlling phototactic behaviour by *D. discoideum* slugs. *Cell* **23**, 799–807.

Francis, D. W. (1964). Some studies on phototaxis of *Dictyostelium*. *J. cell. comp. Physiol.* **64**, 131–138.

Hazelbauer, G. L., ed. (1978). *Taxis and Behavior, Receptors and Recognition*, series B, vol. 5. London: Chapman and Hall.

Inouye, K. & Takeuchi, I. (1979). Analytical studies on migrating movement of the pseudoplasmodium of *Dictyostelium discoideum*. *Protoplasma* **99**, 289–304.

Koshland, D. E. (1979). A model regulatory system: bacterial chemotaxis. *Physiol. Rev.* **59**, 811–862.

Mullens, I. A. & Newell, P. C. (1978). cAMP binding to cell surface receptors of *Dictyostelium*. *Differentiation* **10**, 171–176.

North, M. J. & Williams, K. L. (1978). Relationship between the axenic phenotype and sensitivity to ω-aminocarboxylic acids in *Dictyostelium discoideum*. *J. gen. Microbiol.* **107**, 223–230.

Poff, K. L. & Loomis, W. F. (1973). Control of phototactic migration in *Dictyostelium discoideum*. *Expl Cell Res.* **82**, 236–240.

Raper, K. B. (1940). Pseudoplasmodium formation and organisation in *Dictyostelium discoideum*. *J. Elisha Mitchell scient. Soc.* **56**, 241–282.

Smith, E. & Williams, K. L. (1980). Evidence for tip control of the 'slug/fruit' switch in slugs of *Dictyostelium discoideum*. *J. Embryol. exp. Morph.* **57**, 233–240.

Smith, E. & Williams, K. L. (1981). The age-dependent loss of cells from the rear of a *Dictyostelium discoideum* slug is not tip controlled. *J. Embryol. exp. Morph.* **61**, 61–67.

Stenhouse, F. O. & Williams, K. L. (1981). Investigation of cell patterning in the asexual fruiting body of *Dictyostelium discoideum* using haploid and isogenic diploid strains. *Differentiation* **18**, 1–9.

Whitaker, B. D. & Poff, K. L. (1980). Thermal adaptation of thermosensing and negative thermotaxis in *Dictyostelium*. *Expl Cell Res.* **128**, 87–93.

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