TRANSITIONS IN DICTYOSTELIUM DISCOIDEUM
BEHAVIOUR: INFLUENCE OF CALCIUM AND
FLUORIDE ON SLUG PHOTOTAXIS AND
THERMOTAXIS

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SUMMARY
Phototaxis and thermotaxis by slugs of Dictyostelium discoideum show transitions that result in
bimodality in phototaxis and temperature-dependent orientation up or down temperature gradients. New steps in the sensory transduction chain for these kinds of behaviour are elucidated from studies using inorganic salts (Ca\(^{2+}\), EGTA, KF) and several mutants. KF enhances bimodality of phototaxis, improves the accuracy of thermotaxis, and affects the transition temperatures from positive to negative thermotaxis. Changing the Ca\(^{2+}\) concentration has effects on both phototaxis and thermotaxis. At low Ca\(^{2+}\) concentrations phototaxis and thermotaxis are enhanced. In the presence of EGTA or high concentrations of Ca\(^{2+}\) phototaxis becomes bimodal, thermotaxis is impaired and spontaneous turning is suppressed. These results obtained by changing Ca\(^{2+}\) concentrations are analogous to those obtained previously with sensory transduction mutations, which coordinately affect phototaxis and thermotaxis.

INTRODUCTION
The behaviour of multicellular Dictyostelium discoideum slugs was once thought to be simple - slugs moved directly towards light (Bonner, Clarke, Neely & Slifkin, 1950; Poff & Loomis, 1973) and 'up' heat gradients (Bonner, Clarke, Neely & Slifkin, 1950; Poff & Skokut, 1977). However, recent results are more complex. Phototaxis is bidirectional, i.e. slugs move at certain angles at either side of the light source, with the angle of orientation (\(\alpha\)) affected by mutation and STF (Slug Turning Factor) levels (Fisher & Williams, 1981). In wild-type strains \(\alpha\) is so small that orientation seems to be unidirectional. Thermotaxis exhibits two transitions. At low temperatures slugs move towards the cold (Whitaker & Poff, 1980; Fisher & Williams, 1982). At intermediate temperatures slugs move up the heat gradient and become oriented most accurately, close to the growth temperature (Whitaker & Poff, 1980; Fisher & Williams, 1982). At high temperature a second transition towards the cold is observed (Fisher & Williams, 1982; and this paper).

From studies on behavioural mutants it is clear that phototactic and thermotactic pathways converge (Fisher, Smith & Williams, 1981; Fisher & Williams, 1982; mutants of Schneider, Fontana & Poff, 1982; discussed by Fisher, Dohrmann & Williams, 1983b). In this paper we expand the sensory transduction chain by
demonstrating that inorganic ions, notably calcium and fluoride have specific effects on phototaxis and thermotaxis.

**MATERIALS AND METHODS**

**Strains and culture of D. discoideum**

Strain X22 and phototaxis mutants HU120 and HU409 have been described elsewhere (Fisher & Williams, 1982). Stocks of *D. discoideum* were stored as spores on silica gel. Amoebae were grown in association with *Klebsiella aerogenes* on nutrient SM-agar at 21 ± 1 deg. C in the dark (Williams & Newell, 1976).

**Phototaxis and thermotaxis experiments**

Previously described methods were used to study slug phototaxis and thermotaxis (Fisher et al. 1981; Fisher & Williams, 1982). Amoebae were washed free of bacteria and resuspended in Bonner’s salt solution (0.6 g/l NaCl, 0.75 g/l KCl, 0.4 g/l CaCl₂·2H₂O), at 10⁷/ml or 2.5 × 10⁷/ml; 20 µl of amoebal suspension was placed in an 1 cm² origin in the centre of an agar plate containing 35 ml water agar, and incubated for either 48 h at 21 ± 1 deg. C (phototaxis) or for 72 h (thermotaxis). For thermotaxis experiments, eight plates were placed in a temperature gradient of 0.2 deg. C/cm with temperatures at the centres of the plates ranging from 14°C to 28°C. The heat bar was sufficiently large to hold 11 such series of plates, so replicates and appropriate controls could be run in each experiment. All experiments were performed on water agar rather than on charcoal/water agar (see Fisher et al. 1981). In most experiments low-ash noble agar (Difco) instead of Difco Bacto agar was used in order to minimize extraneous metal ions (see Materials, below). At the end of each experiment slime trails were transferred to PVC discs and stained with Coomassie Blue (Fisher et al. 1981). We found that the visibility of the trails was greatly improved after the discs were destained with hot water or 10% acetic acid. Destaining removed the dye from the disc itself but did not affect staining of the trails.

**Spontaneous orientation**

Experiments to measure spontaneous turning by slugs migrating in the dark in the absence of a temperature gradient were conducted as described elsewhere (Fisher, Grant, Dohrmann & Williams, 1983a).

**Analysis of behaviour**

The principles of the data analysis have been explained in detail in earlier reports (Fisher et al. 1981, 1983a). Trails were digitized using a digitizing tablet (Summagraphics) connected to a VAX 11 computer. Estimates of the accuracy of phototaxis and thermotaxis (k) and of spontaneous ‘noise’ in slug steering (rad²/h) were obtained using directional statistics. The figures displayed in this paper were prepared by digitizing trails and plotting them from a common origin using a VAX 11 plot program. The trails were plotted so that the direction towards the point light source or warmth (in thermotaxis experiments) was towards the top of the figure. In some cases, particularly at high cell densities, not all trails were plotted but those trails plotted were representative of the behaviour (i.e. k was the same as that calculated for the whole population).

**Materials**

Ethylene glycol bis(β-aminoethylether)-N,N'-tetraacetic acid (EGTA), CaCl₂, NaF and KF were analytical grade reagents from Sigma.

Sterile stock solutions and sterile agar were made up with deionized water (18.5 MΩ resistance, 10 µS-Ca²⁺). Appropriate volumes of stock solutions were added to water agar immediately before pouring the plates. Our standard water agar was made with Bacto agar (Difco). We observed (see Results) that this had some effects on behaviour different from those seen when low-ash noble agar (Difco) was used. At present we cannot explain the differences that are almost certainly not due to
Ca$^{2+}$. The Ca$^{2+}$ concentrations in the water agar were 0.6 mM (Bacto) and 0.4 mM (noble), respectively. However, all experiments involving external Ca$^{2+}$ were done with noble agar. Ca$^{2+}$ was determined by inductively coupled plasma atomic emission spectrometry (Jarrell-Ash, ICAP 9000) and the measurements were performed by Dr R. Röhl.

**RESULTS**

*Fluoride accentuates bimodal phototaxis*

Fluoride had a pronounced effect on the phototaxis of the wild type (X22) and most (e.g. HU120) but not all (e.g. HU409) mutants of *D. discoideum* examined (see Fig. 1 for 7.5 mM-KF). (In all experiments reported here, potassium fluoride was used,

![Fig. 1](image_url)

Fig. 1. Phototaxis by slugs of strain X22 and phototactic mutants HU120 and HU409 in the presence and absence of KF; 2 × 10^6 amoebae were placed on an 1 cm^2 origin in the centre of a water/noble agar plate ±7.5 mM-KF. The light source was at the top of the page, and the trails were plotted from a common origin (see Materials and Methods).
but sodium fluoride produced the same effects.) Strain X22 was detectably bimodal at KF concentrations above 5 mM and exceedingly bimodal at 10 mM and higher concentrations, while aggregation and slug speed were scarcely affected. At 20 mM-KF not all X22 cells aggregated and aggregates that were formed did not migrate. At low concentrations (approx. 1 mM) KF actually improved phototaxis although this was not a marked effect because under standard conditions wild-type strains (e.g. X22) are not noticeably bimodal.

**Fluoride enhances thermotaxis and alters the transitions to negative thermotaxis**

Concentrations of 5 mM to 15 mM-KF, which produced marked bimodal phototaxis (see Fig. 1 for 7.5 mM-KF), improved the accuracy of thermotaxis in most strains and shifted the transitions away from the growth temperature (see Figs 2 and 3 for X22 slugs that migrated on normal Bacto/water agar ± KF). Fig. 2 shows the effect of KF (5 mM) over the whole range of temperatures at which slugs can migrate. In Fig. 3, individual trails of slugs migrating at 16°C and at 20°C in the presence or absence of 7.5 mM-KF were plotted. These particular temperatures were chosen for illustration, since at 16°C control slugs still migrated towards the cold, whereas slugs on KF/agar had already undergone the transition from negative to positive thermotaxis. At 20°C both control slugs and slugs on KF were moving towards the warmth, and here it is clearly seen that orientation was improved in the presence of KF. The accuracy of

![Fig. 2. Influence of KF on the accuracy of thermotaxis of X22 slugs. The experiments were performed on normal water agar (---) or on normal water agar containing 5 mM-KF (-----); 4 x 10⁶ amoebae were placed on a 1 cm² origin. The temperature gradient was 0.2 deg. C/cm, and for each test eight plates were assayed at temperatures ranging from 14°C to 28°C. Trails were plotted as in Fig. 1.](attachment:image)
orientation on KF/agar was even greater at 18°C than at 16°C, while at this temperature the control slugs were close to the transition from negative to positive thermotaxis (as can be seen in Fig. 2).

**Ca**²⁺ and slug phototaxis

Because of the significant role of Ca²⁺ in photosensory transduction of other organisms (Hildebrand, 1980) as well as in chemotaxis/aggregation of *D. discoideum* amoebae (Gerisch & Malchow, 1976; Devreotes, 1982), we examined slug behaviour in the presence of Ca²⁺ and EGTA, as the most specific Ca²⁺ chelator. Furthermore, EGTA was used to see if the effect of KF on slug behaviour could be explained in terms of Ca²⁺ sequestration: if it did mimic KF, then the effect of KF would most probably be due to lowered Ca²⁺ concentrations (by precipitation of highly insoluble calcium fluoride). Like KF, EGTA caused bimodal phototaxis in essentially all strains tested (e.g. X22, ±3 mM-EGTA, Fig. 4A, B). At concentrations above 5 mM-EGTA, aggregation was severely impaired in most strains. However, unlike KF, low concentrations of EGTA did not lead to improved orientation.

![Fig. 3](image-url)  
*Fig. 3. Migration patterns of X22 slugs in a thermogradient in the absence and presence of KF; 2 x 10⁶ amoebae were placed in the origins of normal water agar plates with or without 7.5 mM-KF. Temperatures at the origins were 16°C or 20°C. The temperature gradient was 0-2 deg. C/cm.*
Fig. 4. Phototaxis of X22 slugs in the absence and presence of EGTA and CaCl₂; 2 × 10⁶ amoebae were placed on a 1 cm² origin on noble agar. The agar contained no salts (A, C) or 3 mM-EGTA (B), 2-5 mM-CaCl₂ (D) or 12-5 mM-CaCl₂ (E), respectively. Other details were as in Fig. 1.

Conversely, adding low amounts of Ca²⁺ (1-2-5 mM) led to improved (decreased bimodality) phototaxis (Fig. 4D), which was more pronounced if noble agar was used (see below). At higher concentrations of Ca²⁺ (7-5-15 mM) the accuracy of phototaxis was slightly decreased (Fig. 4E). Concentrations of Ca²⁺ higher than 20 mM often reduced the number of aggregates and resulted in failure of slugs of strain X22 (and most strains tested) to migrate. Thus, the results with Ca²⁺ showed that there was an optimal Ca²⁺ concentration (approx. 1 mM) for slug phototaxis.

**Ca²⁺ and slug thermotaxis**

Although EGTA and high Ca²⁺ produced effects similar to KF in phototaxis (Fig. 1 versus Fig. 4B, E), their effects on thermotaxis were different (see Fig. 3 versus Fig. 5). The accuracy of thermotaxis was decreased by EGTA and high Ca²⁺ concentrations (Fig. 6), and the transitions were shifted towards the growth temperature, i.e. 21 °C (and not away from it as with KF). Migration of slugs at high temperature was greatly affected in the presence of EGTA (Fig. 5). The accuracy in thermotaxis was improved slightly at low Ca²⁺ concentrations (0-5-1 mM, data not shown). These findings reinforce the observations on phototaxis that slug behaviour was optimal at a Ca²⁺ concentration in the range from 0-5 to 2 mM.

**Ca²⁺ effects on unstimulated behaviour**

In a recent report we showed that KF markedly suppressed spontaneous 'noise' in
Fig. 5. Thermotaxis of X22 slugs in the absence and presence of Ca²⁺ and EGTA. The experiment was performed as in Fig. 3 except that noble agar, without or with 12.5 mM Ca²⁺ or 1 mM EGTA, was used. Temperatures at the origins were 16°C, 20°C and 26°C. Trails were plotted as in Fig. 1.

slug orientation in the dark in the absence of a temperature gradient without affecting the slug speed (Fisher et al. 1983a). Spontaneous turning on noble agar in the presence of 1 mM-EGTA was reduced by up to 50% in three experiments (steering noise (rad²/h) in controls: 0.35 ± 0.08, 0.52 ± 0.13, 0.27 ± 0.03; with 1 mM-EGTA: 0.22 ± 0.04, 0.26 ± 0.07, 0.13 ± 0.03). We observed a smaller decrease in spontaneous turning in the presence of 15 mM-Ca²⁺. Steering noise in the presence of 1 mM and 2.5 mM-Ca²⁺ was similar to control values. We conclude that some spontaneous signals arise ‘upstream’ from Ca²⁺ in the sensory transduction chain in D. discoideum slugs.

Choice of agar

A surprising finding was that the type of agar used was critical, especially in thermotaxis experiments. When slugs migrated on normal water agar, all slugs were oriented towards the cold at 16°C (see control slugs in Fig. 3), whereas on noble agar
about half of the slugs were migrating in each direction at this temperature (see control slugs in Fig. 5). Furthermore, with noble agar a second transition in orientation (down the heat gradient) was observed at high temperature (Figs 5, 6). This transition was not seen reproducibly when X22 thermotaxis was performed on normal Difco agar. The effects were not due to a difference in the Ca\(^{2+}\) content in the agar (see Materials and Methods).

**DISCUSSION**

The experiments reported here on the behaviour of *D. discoideum* slugs in the presence of Ca\(^{2+}\) and EGTA establish a role for Ca\(^{2+}\) in the sensory transduction chain. Like several mutations studied previously (Fisher & Williams, 1982), Ca\(^{2+}\) affected both accuracy of orientation and transitions in phototaxis and thermotaxis. As we have discussed previously for sensory transduction mutations (Fisher & Williams, 1982) these effects can be economically explained via a single mechanism, rather than multiple mechanisms affecting phototaxis and thermotaxis separately. The site(s) of action of Ca\(^{2+}\) must be before or in the region of the sensory transduction chain at which the angle of phototactic orientation and changes in the direction of thermotaxis are set (Fisher, 1981; Williams, 1982; Fisher *et al.* 1983b), since we have shown here that Ca\(^{2+}\) affected these processes (Figs 4, 5).

Ca\(^{2+}\) has been established as an important effector molecule for (photo)sensory
transduction processes in many lower and higher organisms (Rasmussen et al. 1975; Hildebrand, 1980). In most of the systems studied an optimal extracellular Ca\(^{2+}\) concentration has been observed (e.g. in leucocyte chemotaxis; Sha'afi & Naccache, 1981), and our observations in *D. discoideum* are analogous. It is clear that Ca\(^{2+}\) also plays an essential role in the early stages of *D. discoideum* development, i.e. in aggregation of amoebae, which involves chemotactic responses to a self-generated cAMP gradient (Mason, Rasmussen & Dibella, 1971; Gerisch & Malchow, 1976; Devreotes, 1982). For example, it has been found that chemotaxis and aggregation were severely impaired at very low (in the presence of EGTA) and very high (>150 mM) Ca\(^{2+}\) concentrations (Loomis, Klein & Brachet, 1978; Maeda, 1970). In a recent study more precise techniques were used to investigate the influence of Ca\(^{2+}\) in amoebal chemotaxis (Malchow, Böhme & Gras, 1982). A concentration of 1 mM-Ca\(^{2+}\) was found to enhance chemotaxis by amoebae to cAMP maximally, although higher concentrations were not tested. We have shown here that slug phototaxis and thermotaxis were optimal at a Ca\(^{2+}\) concentration of about 0.5-2 mM, varying slightly between single experiments and depending on the particular strain tested.

Ca\(^{2+}\) is clearly involved in morphogenesis as high Ca\(^{2+}\) (up to 120 mM) increases the proportion of stalk cells in *D. discoideum* fruiting bodies (Maeda, 1970) while EGTA (3 mM) causes an increase in the percentage of prespore cells in the migrating slug (Krefft, Voet, Mairhofer & Williams, 1983). Since it has been reported that fluoride increases the proportions of spore cells in mature fruiting bodies (Maeda, 1970) and of prespore cells in slugs (Durston & Vork, 1977), we began studies on the influence of KF on slug behaviour. KF clearly had a profound effect. Unlike all mutations and other effectors examined (e.g. STF, Ca\(^{2+}\)), KF had opposite effects on slug phototaxis and thermotaxis. The fact that cells aggregated normally and thermotaxis was enhanced argues strongly against a non-specific poison effect of KF. The fluoride effect was not due to Ca\(^{2+}\) chelation (studied here using EGTA). Although both EGTA (up to 5 mM) and KF (>5 mM) led to bimodal phototaxis (Figs 1, 4) and suppression of spontaneous turning, thermotaxis was enhanced by KF (Figs 2, 3) but impaired by EGTA (Figs 5, 6).

The action of fluoride on multiple sites of the sensory pathway cannot of course be excluded, but if signals from the photoreceptor and thermoreceptor are biochemical opposites (Poff & Loomis, 1973; Fisher et al. 1981), KF could also act on a single element of the sensory transduction chain after convergence of photo-thermo-transduction. A possible site of action would be activation of adenylate cyclase. It is noteworthy that several groups have proposed a role for cAMP as an extracellular chemical signal in slug behaviour (Maeda, 1977; Durston, Vork & Weinberger, 1979; Matsukuma & Durston, 1979; Kessin, 1982; Fisher et al. 1983b). However, while fluoride is a well-established activator of hormone-dependent adenylate cyclases (Rose & Gilman, 1980; Limbird, 1981), stimulation of adenylate cyclase activity by fluoride has not been observed using membranes from aggregating amoebae of *D. discoideum* (Klein, 1976). On the other hand, we have measured elevated cAMP levels in slugs that migrated on KF/agar (Dohrmann, Brüderlein & Williams, unpublished data).
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