Phototaxis during the slug stage of *Dictyostelium discoideum*: a model study

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During the slug stage, the cellular slime mould *Dictyostelium discoideum* moves towards light sources. We have modelled this phototactic behaviour using a hybrid cellular automata/partial differential equation model. In our model, individual amoebae are not able to measure the direction from which the light comes, and differences in light intensity do not lead to differentiation in motion velocity among the amoebae. Nevertheless, the whole slug orientates itself towards the light. This behaviour is mediated by a modification of the cyclic AMP (cAMP) waves. As an explanation for phototaxis we propose the following mechanism, which is basically characterized by four processes: (i) light is focused on the distal side of the slug as a result of the so-called 'lens-effect'; (ii) differences in luminous intensity cause differences in NH_3 concentration; (iii) NH_3 alters the excitability of the cell, and thereby the shape of the cAMP wave; and (iv) chemotaxis towards cAMP causes the slug to turn. We show that this mechanism can account for a number of other behaviours that have been observed in experiments, such as bidirectional phototaxis and the cancellation of bidirectionality by a decrease in the light intensity or the addition of charcoal to the medium.

Keywords: *Dictyostelium*; phototaxis; biological models; movement; ammonia; cyclic AMP

1. INTRODUCTION

Upon starvation, individual amoebae of the microorganism *Dictyostelium discoideum* start to aggregate and form migrating multicellular slugs. During the slug stage prestalk cells group in the anterior part of the slug, whereas prespore cells end up in the posterior part. Each slug culminates in a fruiting body consisting of a globule of spore cells on a slender stalk. The motion of amoebae is orchestrated by waves of cyclic AMP (cAMP), which are formed by a combination of a pulsatile cAMP excretion and a cAMP-mediated cAMP response. This is accompanied by a chemotactic response to cAMP.

Migrating slugs are orientated by light (phototaxis), temperature gradients (thermotaxis), pH differences (acidotaxis), and wind (rheotaxis). The orientation towards light (as well as towards the other cues) leads to migration towards the soil surface, which is beneficial for spore dispersal (Bonner *et al*. 1985).

A slug starts to turn towards a light source after 10 min of irradiation by light coming from one side (Yumura *et al*. 1992). Phototaxis and thermotaxis must use different pathways, because even light intensities that are too low to cause a local increase in temperature elicit a response. During phototaxis the slug functions as a lens that focuses light on the side opposite to the light source. This so called 'lens-effect' was first postulated by Buder (1920) for *Phycomyces*, and was shown by Francis (1964) to be the mechanism in *D. discoideum* (see also Poff *et al*. 1986). Several researchers have confirmed this lens-effect by, for example, illuminating only half of the

slug (Francis 1964), placing the slug in mineral oil (Bonner & Whitfield 1965), or introducing neutral red into the cells (Häder & Burkart 1983). In all these cases it has been found that a slug orientates away from the light, because light is prevented from focusing on the distal side, but instead illuminates the proximal side more, due to absorption.

It is not yet fully understood how the light signal is transformed into tactic behaviour. Bonner *et al*. (1988) argued that ammonia (NH_3) could play an important role in this process, because they found that, on the one hand, light causes slugs to produce NH_3, whereas on the other hand, the response to light diminishes when the slugs are completely surrounded by NH_3. Several authors have reported negative chemotaxis away from NH_3, and have shown that the amount of NH_3 produced by slugs is sufficient for such a motion (Bonner *et al*. 1986; Feit & Sollitto 1987; Kosugi & Inouye 1989; Yumura *et al*. 1992).

Francis (1964) suggested that light may speed up cell motion, and that this could be a sufficient explanation for phototaxis. This led to the hypothesis that light stimulates the local production of NH_3, which in turn stimulates the cells to move faster, forcing the slug to turn towards the light (Bonner *et al*. 1986, 1988). However, there is still a dispute about whether NH_3 does indeed speed up cell motion, because more detailed studies reported that average slug speed may be unaffected by NH_3 or light, and not even a transient increase could be detected (Smith *et al*. 1982; Fisher 1997). Furthermore, Davies *et al*. (1993) did not find that NH_3 caused any change in chemotaxis towards cAMP.

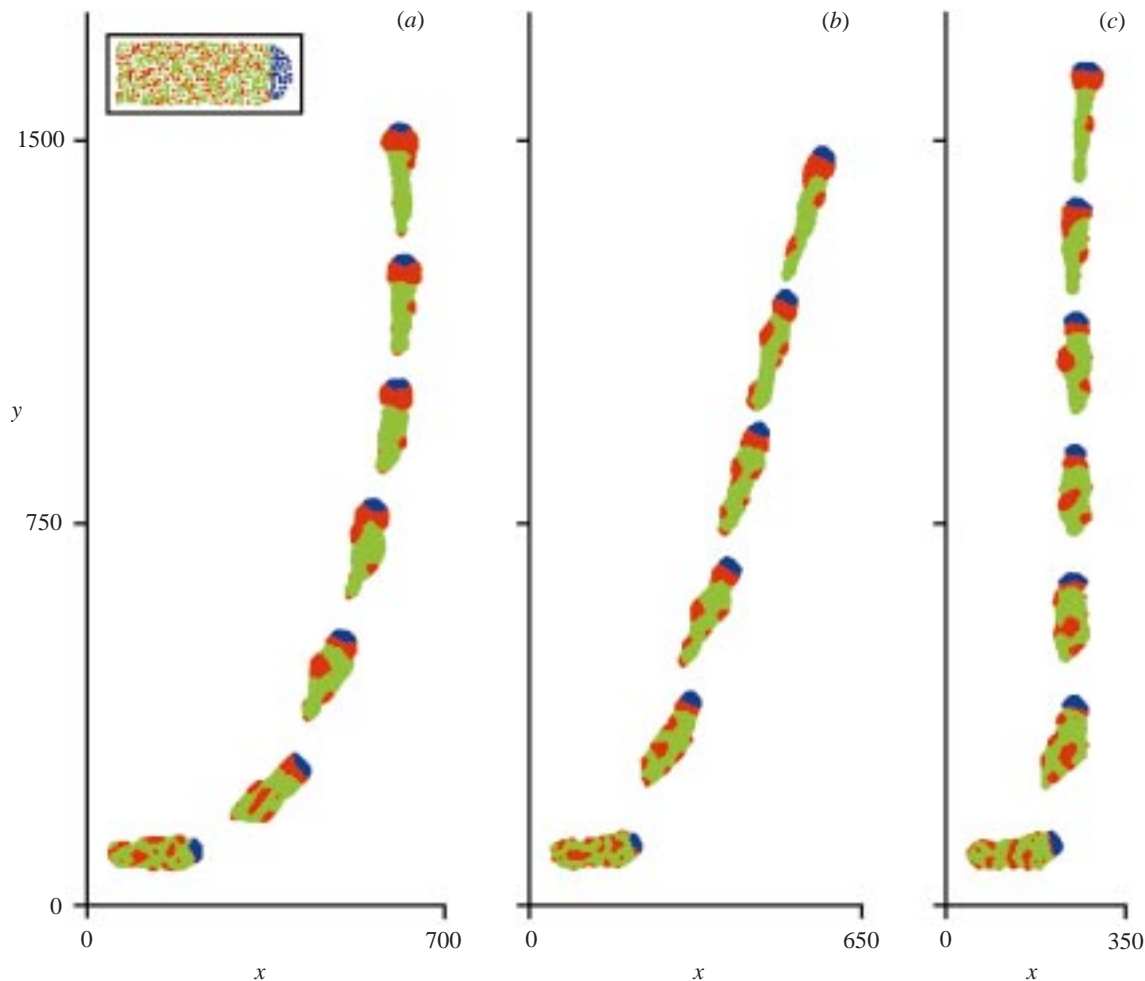During phototaxis the trails of slugs belonging to certain mutant strains can become unstable, bidirectional

Figure 1. Time sequences from simulations of motion during the slug phase of *D. discoideum*. (*a*) A simulation of phototaxis, with light radiating from top to bottom. (*b*) A simulation of a mutant with bidirectional phototaxis. (*c*) A simulation of the same mutant surrounded by NH_3-absorbing charcoal. The inset in (*a*) shows the initial distribution for all three simulations, enlarged two times, with a tip consisting of oscillatory prestalk cells and a body consisting of 40% prestalk and 60% prespore cells. The first frames show the state after 15 min (9000 time-steps). Successive frames with intervals of (*a*) and (*c*) 1 h 15 min (45 000 time-steps); and (*b*) 1 h 30 min (54 000 time-steps). There are 430 amoebae. One time-step (solution of the partial differential equations) corresponds to *ca*. 0.1 s and one grid point to 5 μm. Cell types are $\tau \in \{a, t, p\}$ where a is oscillatory prestalk (blue), t is prestalk (red), and p is prespore (green). Bond energies are $\mathcal{J}_{a,a} = 3$, $\mathcal{J}_{t,t} = 5$, $\mathcal{J}_{p,p} = 7$, $\mathcal{J}_{a,M} = 7$, $\mathcal{J}_{t,M} = 8$, $\mathcal{J}_{p,M} = 9$, $\mathcal{J}_{a,t} = 6$, $\mathcal{J}_{t,p} = 8$ and $\mathcal{J}_{a,p} = 9$. $T = 2$, $V = 30$, $\lambda = 0.6$, and $\mu = 200$. The parameters used for the partial differential equations are $D_c = 1$, $C_1 = 20$, $C_2 = 3$, $C_3 = 15$, $\varepsilon_1 = 0.5$, $\varepsilon_2 = 0.0589$, $\varepsilon_3 = 0.5$, $k = 3.5$, $d_c = 0.05$, $c_0 = -0.3$, $D_n = 15$, $d_{n_{in}} = 0.01$, and $b = 0.1$. $p = 1$ in simulation (*a*), and $p = 4$ in simulations (*b*) and (*c*). In simulation (*c*) $d_{n_{out}} = 0.3$. The partial differential equations are solved by the explicit Euler method (with time-step equal to 0.01 and space-step equal to 0.37). The axes are given in grid points.

(Fisher & Williams 1981), or even multidirectional (Fisher *et al*. 1985). We specifically focus on the bidirectional mutants. At low light intensities their bidirectionality decreases, or even disappears (Poff *et al*. 1986). Moreover, even at high light intensities the trail of such mutants can be made more stable and shown to deviate less from the direction of light if activated charcoal is added to the substrate (Fisher & Williams 1981; Haser & Häder 1992). There are some indications that this reaction is related to the absorption of NH_3 by the charcoal (Bonner *et al*. 1986; Haser & Häder 1992). In general, slugs orientate towards a piece of charcoal, but if the charcoal is first saturated with NH_3, it is no longer capable of attracting slugs (Bonner 1993). This is yet another indication that the main effect of the charcoal is to absorb NH_3.

In this paper we model phototaxis in terms of an NH_3-mediated modification of the shape of cAMP waves only;

no differences in chemotactic response were assumed. We show that this modification is sufficient to account not only for phototaxis, but also for a number of other behaviours which are observed in connection with phototaxis.

2. THE MODEL

There have been several models describing slug migration (Odell & Bonner 1986; Williams *et al*. 1986; Umeda 1989; Savill & Hogeweg 1997; Bretschneider *et al*. 1995; Marée *et al*. 1999). However, these models were not used to describe phototaxis. In this study we have extended our model for thermotaxis (Marée *et al*. 1999), a hybrid cellular automata (CA)/partial differential equation model, which was formulated by Savill & Hogeweg (1997) to describe the development from single cells to crawling slugs. In these hybrid models a CA is used to
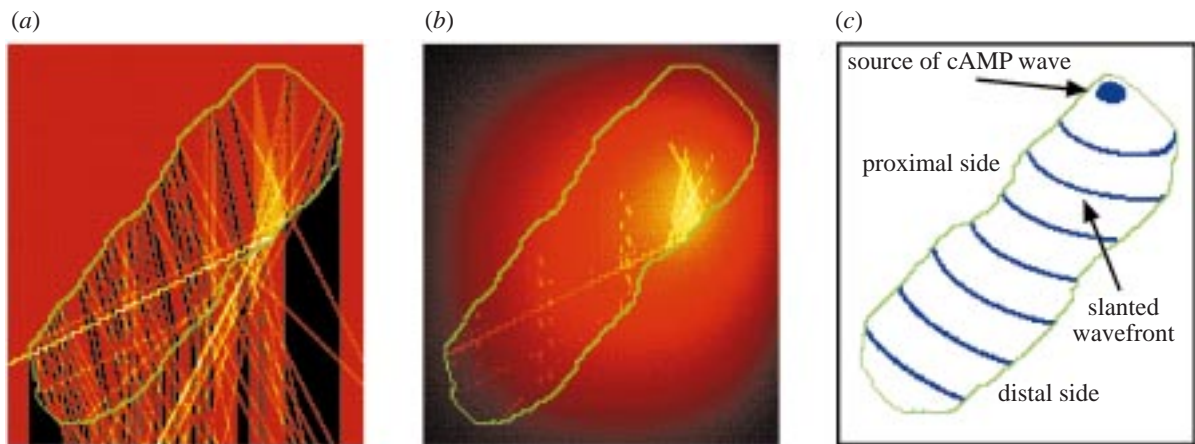
Figure 2. (*a*) Snapshot of the luminous intensity distribution during phototaxis. Light is focused on the distal side due to the lens effect. (*b*) Snapshot of the distribution of NH_3. Lowest concentrations are found on the proximal side. Different levels of the luminous intensity and the corresponding NH_3 concentration are indicated by a colour ramp from dark red (low values) to bright yellow (high values). All parameters are as described in the legend to figure 1. (*c*) A schematic diagram that shows some of the basic properties of the cAMP wave during phototaxis. The blue lines show the wave shape at different locations in the slug.

represent individual amoebae and light, and partial differential equations to model diffusible chemicals. The models are based on a special CA model-formalism, developed by Glazier & Graner (1993). The strength of this formalism is that amoebae are represented as a group of connected automata instead of point-like objects. Therefore amoebae can slide past one another and deform themselves and adjoining amoebae by means of small changes in their boundaries. Very recently, Jiang *et al.* (1998) have used the same formalism to describe tip formation during the mound stage of *D. discoideum*.

In our previous study, on thermotaxis in *D. discoideum* slugs, we showed that taxis can develop as a result of differences in the excitability of the amoebae. These differences in excitability cause the cAMP waves to change shape, which, via the chemotaxis towards cAMP, causes the slug to turn. We have made several extensions to our previous model: the CA has been extended with a description of irradiation and refraction, an extra partial differential equation has been added to describe the NH_3 dynamics, and the cAMP-modulated cAMP response has become dependent on the NH_3 concentration. Since the relationship between NH_3 and cell speed is still under dispute, we have decided to omit from our model any differences in chemotaxis between cell types or due to NH_3.

We study phototaxis in two-dimensional (2D) slugs. This is not a limitation of our model, because Bonner (1998) has recently managed to develop an experimental method for producing migrating 2D (one cell thick) slugs which share most basic properties with normal three-dimensional (3D) slugs.

Each amoeba occupies about 30 automata in the CA, and has an associated label τ, which indicates whether the cell type is prespore, prestalk or oscillatory prestalk ($\tau \in \{p, t, a\}$). Each automaton that is part of an amoeba's boundary has a number of dimensionless free energy bonds. The magnitude of these bonds depends on the cell types they connect. The energy bonds are given by $\mathcal{J}_{\tau_1,\tau_2} > 0$, where τ_i are the types of the two amoebae. The bond energy between an amoeba and the medium is

given by $\mathcal{J}_{\tau,M}$. The total free energy of an amoeba is given by

$$H_\sigma = \sum \frac{\mathcal{J}_{\text{cell,cell}}}{2} + \sum \mathcal{J}_{\text{cell,medium}} + \lambda(v - V)^2, \qquad (1)$$

where v is the volume of the cell, V the target volume, and λ the inelasticity. The final term ensures that the volume of a cell remains close to V. Minimization of the free energy of the amoebae causes deformation of the boundaries. The probability that the boundary will be deformed is either unity if $\Delta H < -0.8$, or $\exp -[(\Delta H + 0.8)/T]$ if $\Delta H \geqslant -0.8$.

We choose bond energies so that the amoebae adhere to each other, but also so that, if given the possibility, they will sort themselves into three fairly homogeneous groups. Oscillatory cells adhere together more rigidly than prestalk cells, and the latter adhere together more rigidly than prespore cells. For further details, see Marée *et al.* (1999).

The luminous intensity l is calculated using the ray-tracing technique. For some basics on ray tracing see, for example, Stavroudis (1972). In our computations, for every position along the upper border of the CA a ray is initiated, with a downward ray direction. Light that enters or leaves a slug is refracted. First, one finds the point where the ray intersects the slug surface. At that point the normal to the surface is calculated: after assigning the value 0 or 1 to automata outside or inside the slug and taking the eight-neighbourhood into account, we compute the local gradient. The direction of the gradient is used as the surface normal. Knowing the surface normal and the angle of incidence, which is the angle between the incident ray and the surface normal, the direction of the refracted ray is determined using Snell's law. We use a refractive index of 1.369, which is the value of the refractive index for *D. discoideum*, as measured by Häder & Burkart (1983). Next, one finds the point of intersection of the refracted ray with the slug surface, and so on. To map the ray, which can move not only horizontally and vertically, but in any direction, onto

the discrete CA, we use Bresenham's line algorithm (Bresenham 1965). To obtain the luminous intensity values at each point, we add up the intensities of all individual rays passing through it. By default, the intensity of an individual ray is set to unity, which we refer to as the default light intensity. To change the intensity of the light source, we simply change the intensities of the incoming rays.

Three partial differential equations are used to describe the cAMP and NH$_3$ dynamics. The cAMP dynamics can be described reasonably well in a quantitative way by two variable simplified equations of the FitzHugh–Nagumo (FHN) type. For some basic background on FHN-type models we refer to Grindrod (1996). Such a description reproduces the overall characteristics of cAMP waves such as refractoriness and curvature relation. The main advantage of these models is their simplicity, and therefore their capacity to connect the effects visible in these models with basic properties of the cAMP signalling in *D. discoideum*. FHN-type models are appropriate for a preliminary qualitative study of the behaviour but, of course, are not adequate for a detailed quantitative study.

Here we investigate the basic effects of heterogeneity in excitability, but we are not concerned with the details of cAMP signalling. So for our present purposes, the FHN models are preferred.

For this study we used the following equations, in which c represents cAMP concentration, and r refractoriness of the cells. The third partial differential equation describes the NH$_3$ concentration:

$$\frac{\partial c}{\partial t} = D_c \Delta c - f(c,\tau,n) - r,$$
$$\frac{\partial r}{\partial t} = \varepsilon(c)(kc - r),$$
$$\frac{\partial n}{\partial t} = D_n \Delta n + g(l) - d_{n_{in}} n, \qquad (2)$$

with $f(c,\tau,n) = C_1 c$ when $c < c_1$; $f(c,\tau,n) = -C_2 c + a(\tau,n)$ when $c_1 \leqslant c \leqslant c_2$; $f(c,\tau,n) = C_3(c-1)$ when $c > c_2$, and $\varepsilon(c) = \varepsilon_1$ when $c < c_1$; $\varepsilon(c) = \varepsilon_2$ when $c_1 \leqslant c \leqslant c_2$; and $\varepsilon(c) = \varepsilon_3$ when $c > c_2$. To make the function $f(c,\tau,n)$ continuous, $c_1 = a(\tau,n)/(C_1 + C_2)$, and $c_2 = (a(\tau,n) + C_3)/(C_2 + C_3)$. If the luminous intensity is below the threshold $l_{th} = 3$, $g(l) = 0$, and if $l \geqslant l_{th}$, $g(l) = l$. The small decay $d_{n_{in}}$ is due to the assimilation of ammonia into amino acids (Dunbar & Wheldrake 1997). D_n is much larger than D_c, due to the relatively low diffusion coefficient of cAMP (Dworkin & Keller 1977). Each automaton in the CA is associated with one grid point in the discretized numerical partial differential equations.

Weijer *et al.* (1984) showed that the tip of the slug can be seen as a high-frequency pacemaker, whereas the body of the slug behaves as an excitable medium. This can be modelled using parameter $a(\tau,n)$, which gives a stable limit cycle at negative values, and describes an excitable medium at positive values. Therefore, positive values are used to describe excitable amoebae, whereas negative values are used to describe the oscillatory amoebae in the tip.

Our description of NH$_3$ action is based on the following experimental data. Schindler and Sussman (1979) found

that NH$_3$ inhibits the cAMP-induced cAMP release; Williams *et al.* (1984) established that this is because NH$_3$ blocks intracellular cAMP accumulation by inhibiting the transitory activation of adenylase cyclase in response to the binding of extracellular cAMP to cell surface receptors; and Darcy & Fisher (1990) produced evidence that this inhibition of the cAMP signalling is important in slug behaviour.

Since in the model $a(\tau,n)$ represents the threshold for the cAMP response, we use the parameter a to express the inhibiting effect of NH$_3$ on the cAMP-modulated cAMP response. We do this by specifying an NH$_3$-dependent increase of a. We assume that this increase saturates:

$$a(\tau,n) = a_{0_\tau} + \frac{bn}{1 + n/p}, \qquad (3)$$

with $a_{0_p} = a_{0_t} = 0.1$ and $a_{0_a} = -0.1$.

Outside the amoebae we only implemented a small cAMP decay, d_c, caused by external phosphodiesterase, and when we modelled absorption of NH$_3$ by charcoal, we also implemented an NH$_3$ decay $d_{n_{out}}$:

$$\frac{\partial c}{\partial t} = D_c \Delta c - d_c(c - c_0),$$
$$\frac{\partial n}{\partial t} = D_n \Delta n - d_{n_{out}} n, \qquad (4)$$

where c_0 is the minimal value of variable c. To create either an NH$_3$ gradient or a background NH$_3$ concentration, we used fixed values for the NH$_3$ concentration along the border of the whole CA. The dynamics inside and outside the slug were not modified.

Chemotaxis is incorporated in the model by using the spatial gradient of the cAMP wavefront (Savill & Hogeweg 1997): $\Delta H' = \Delta H - \mu(c_{automaton} - c_{neighbour})$, where $\Delta H'$ is the new change in energy. This makes it more likely that an amoeba will move towards a location with a higher cAMP concentration and less likely that it will move towards one with a lower cAMP concentration. Chemotaxis is only taken into account when cAMP is above a threshold $c_{th} = 0.05$ and refractoriness below a threshold $r_{th} = 0.2$.

Although the process of cell sorting already starts during the mound formation, slugs are still fully capable of generating the prestalk–prespore pattern due to cell sorting (Sternfeld & David 1981). The first cells to sort out are the prestalk A cells, which form the tip and are the source of the cAMP waves (Williams *et al.* 1989; Siegert & Weijer 1992). Therefore in all simulations a slug is initiated with a tip consisting of oscillatory prestalk cells. As a 'worst case scenario' with respect to the cell sorting, the remaining prestalk cells, together with the prespore cells, are distributed randomly in the body of the slug. Please note, however, that slugs which have completed cell sorting show the same phototactic behaviour as slugs which are still in the process of cell sorting.

3. PHOTOTAXIS

We were able to reproduce phototaxis in *D. discoideum*. In figure 1 we demonstrate the phototactic behaviour of our model slugs in the form of a time-lapse image; figure 1a shows a simulation of phototaxis with light
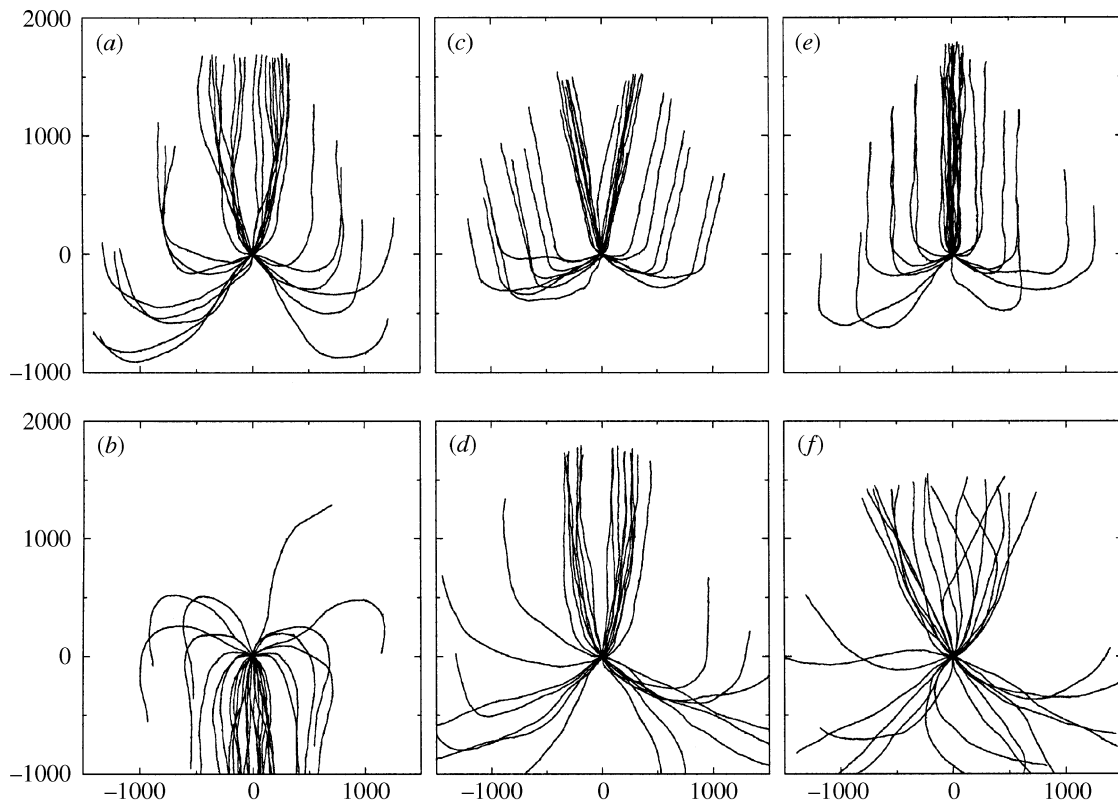
Figure 3. The change in the location of the slug in time, for different model experimental settings. Light radiates from top to bottom; the source of the NH_3 gradient is also at the top. The location at $t = 0$ is set to (0,0), and the simulations lasted for 8 h 20 min (300 000 time-steps). The axes are given in grid points. For each plot 32 simulations were performed. Slugs were initially positioned with respect to the light source at intervals of 22.5°, from 0° onwards. Two simulations per angle were carried out. (*a*) Wild type; default light intensity. (*b*) Wild type; NH_3 gradient of 0.02/grid point. (*c*) Bidirectional mutant; default light intensity. (*d*) Bidirectional mutant; low mean light intensity of 0.8. To analyse the effect of mean light intensities below unity, we needed to use a continuous function for the NH_3 production; $g(l) = l^m/((l_{th} - 0.5)^m + l^m)l$, with $m = 12$. When a mean light intensity of unity is used, simulations using the continuous function are qualitatively indiscernible from the simulations using the original split-function. (*e*) Bidirectional mutant; default light plus charcoal. (*f*) Wild type; default light plus background NH_3 concentration of 2.0. All other parameters are as described in the legend to figure 1.
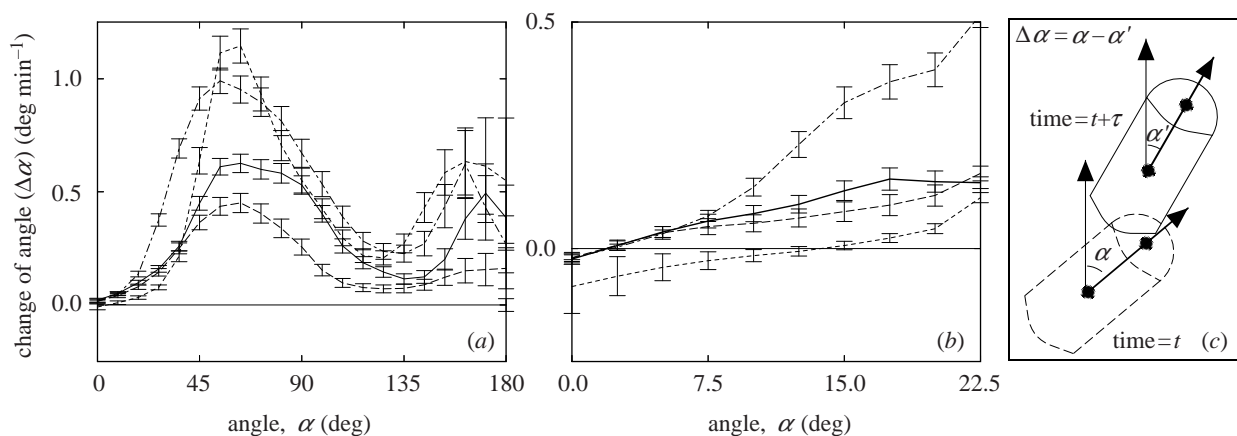

Figure 4. (*a*) Change of angle towards the light source $\Delta\alpha$ versus the position the slug takes up relative to the direction of irradiation α. (*b*) A more detailed graph of the change of angle between 0 and 22.5°. (*c*) Scheme to indicate method used to calculate the change of angle. The various types of line show the change of angle made by the wild type (solid lines), the bidirectional mutant (dotted lines), the mutant at low light intensity (dashed lines), and the mutant surrounded by charcoal (dashed–dotted lines). The means and s.e.m. values (indicated by the bars) were calculated using the data of figure 3. The means and s.e.m. values were calculated at intervals of (*a*) 9°; or (*b*) 2.5°, using a Gaussian smoothing with a standard deviation of 9° and 2.5° again, respectively. The angle α is calculated using the mean location of the oscillatory area relative to the mean location of the rest of the slug. The change of angle $\Delta\alpha$ is calculated using a time difference τ of 2 min 30 s (1500 time-steps).

radiating from top to bottom. The inset shows the initial configuration. The oscillatory prestalk cells are coloured blue, the remaining prestalk cells red, and the prespore cells green. (We have not tried to add pseudo-realism by matching the colours to experimental data from staining experiments.) Initially, the slug moves at an angle of $90°$ relative to the light source, but after 2 h 30 min the angle has decreased to $30°$. Within 7 h the slug has turned completely towards the light. The differences in adhesion between the various cell types are important for the cell sorting (Marée *et al.* 1999), but have little effect on the phototactic behaviour.

In figure 3 the experiments are quantified, each plot showing the slug trails from 32 different simulations. All lines start at the same initial position (0,0), which is visible as a node where all lines originate. Every plot is based on 16 different initial orientations at intervals of $22.5°$.

Figure 3*a* shows normal phototaxis. The final orientation is independent of the initial angle, i.e. in the end the direction of motion is always towards the light. However, when the initial angle is larger than $90°$ turning of the model slug is initially slow.

The mechanism of phototaxis can be described as follows. As a result of the lens effect the light is focused on the distal side, as can be seen in figure 2*a* which shows the distribution of the luminous intensity. Figure 2*b* shows the corresponding NH_3 concentration. Note that although in a snapshot of the luminous intensity discrete effects are clearly visible these do not affect the long-term behaviour, because the slugs' boundary is continuously changing its shape therewith constantly changing the luminous intensity distribution. Because NH_3 inhibits cAMP it causes a longer oscillating period and a lower wave speed in the region of high NH_3 concentration (Marée *et al.* 1999). Hence a region with the shortest oscillating period will be located on the proximal side, which will serve as the source of the cAMP waves (see figure 2*c*).

Hence the cAMP wave starts slanted, and remains slanted when it passes through the region where the light is focused, because in this region there are large differences in NH_3 concentration (and thus in wave speed) between the proximal and distal side. More posteriorly there are only small variations in the NH_3 concentration, and as a consequence the cAMP wave straightens out due to the curvature effect (see figure 2*c*). The slanted cAMP wave, combined with the chemotaxis towards cAMP, leads to the phototactic response: the amoebae move preferably perpendicular to the wavefront, therewith pushing the oscillatory tip into the direction of the light source.

Our model also reproduces experimentally observed negative taxis away from NH_3. Figure 3*b* shows the trails of 32 simulations in a field with the source of NH_3 located at the top, without any light. The negative chemotaxis is clearly visible. Note that individual amoebae in our model do not have any NH_3 taxis. The mechanism is simply that the NH_3 partly diffuses into the slug and causes an internal NH_3 gradient. This again gives rise to the differences in excitability which lead to the tactic behaviour.

Our model reproduces a deviation in the orientation towards light, similar to what is observed in the so-called

bidirectional mutants. We found this behaviour when the decrease in excitability saturates at higher NH_3 concentrations. Figure 1*b* shows a simulation in which we increased the value of p (which gives the half-saturation concentration) four times. The slug still demonstrates pronounced phototaxis, but the final direction of motion is at an angle of $14°$. Figure 3*c* shows the trails of 32 different simulations. This figure clearly shows bidirectional phototaxis. Initially, turning can be slow, but the final deviation from the direction of the light path is independent of the initial angle. If initially the slug is positioned at an angle of $0°$, random variations are amplified by up to $14°$.

In our model and in experiment, bidirectional phototaxis changes back into unidirectional phototaxis at low light intensities (Poff *et al.* 1986). Figure 3*d* shows the trails of the 'bidirectional mutant' from the previous simulations when the light intensity is decreased from unity to 0.8. Now turning takes much longer and the trails are less stable, because the signal is weaker. However, bidirectionality has indeed disappeared. At light intensities stronger than 0.8 bidirectionality can still be observed, although it is weaker; at weaker light intensities phototaxis becomes increasingly less pronounced.

From experiments it is known that charcoal reduces the deviation in slugs' orientation towards light. We found similar results in our model. Figure 1*c* shows such a simulation, in which the bidirectional mutant is surrounded by charcoal. We emulated the addition of charcoal by including the external absorption of NH_3 in equation (4). The slug moves straight to the light, and the bidirectional behaviour has disappeared. Figure 3*e* shows again that this result does not depend on the initial conditions. Even when the initial angle is $180°$, random deviations let the slug turn either to the right or the left.

The mechanism of bidirectional phototaxis can be explained as follows. In equation (3), parameter p gives the NH_3 concentration at which the decrease in excitability is half-saturated, and thereby determines the range of NH_3 concentrations that affect the excitability. If this parameter is increased (and hence the range is enlarged), differences in excitability along the slug become larger. These differences are locally largest between the area on which the light is focused and the area on the other, more excitable, proximal side. The source of the cAMP wave hardly changes position at all, because this location is determined mainly by the lowest NH_3 concentration. However, when the wave reaches the region on which the light is focused, the wave shape changes dramatically, as it moves much more slowly in the region of the light focus on the distal side of the slug than on the proximal side. As a result, the wave becomes much more slanted in this region than is found with normal phototaxis.

The model does not contain explicit forces. However, the adhesion between the amoebae and between the amoebae and the medium create a surface tension (Glazier & Graner 1993), which preserves to a certain extent the shape of the slug. Therefore the chemotactic motion can create force on the slug itself, which has a torque, due to the slanted wavefront. Because the wave is only substantially slanted at a certain distance behind the tip, the oscillatory area is pushed away from the light by

the momentum of this force. This is especially the case when the slug moves at a small angle to the direction of light: the light is focused on an area that is located at a larger distance behind the tip, which creates a longer lever. Hence, at small angles the bidirectional mutant moves away from the light. Note that the effect is caused by differences in motion direction and not by differences in motion speed. In fact, when following individual amoebae, we observe rather wriggling paths of motion. Only the gross activity shows the fixed bidirectional motion.

The absorption by charcoal decreases the NH_3 concentration. This has only a marginal effect on the position with the lowest concentration and thus on the source of the new cAMP wave but reduces the slant of the cAMP wave and thereby the momentum of the force sufficiently to re-establish unidirectional phototaxis. The effect of decreasing the light intensity works via the same principle as the addition of charcoal, namely it creates lower NH_3 concentrations. The main difference between low light intensity and adding charcoal is that at low light intensities the signal becomes weaker, which causes slower turning and more unstable trails, whereas when charcoal is added the signal remains strong, and therefore stability and turning velocity are still very high.

In our last simulations we considered the experimental observation that external NH_3 decreases phototaxis. Figure 3*f* shows the trails of our original unidirectional phototactic slug when surrounded by an NH_3 concentration of 2.0. Obviously, phototaxis diminishes because the high concentration of NH_3 strongly interferes with the phototactic signalling system. However, very high concentrations are needed to suppress phototaxis completely, because it is driven by relative differences in NH_3 concentration and not by absolute values.

Next, using the data from figure 3, we analysed the velocity at which slugs turn towards the light. Figure 4*a* shows the change of angle versus the angle by which the slug deviates from the light source. Figure 4*b* gives a more detailed graph of the change of angle between 0 and 22.5°. To obtain the figure, we took into account that this turning is, to the left and right, symmetrical around the direction of the light path. For normal phototaxis, there is only one stable direction of motion, namely towards the light source. The bidirectional mutant turns much faster because its sensitivity to NH_3 is much higher, but the phototactic force towards the light and the momentum of the force away from the light equilibrate at an angle of 14°, which is clearly visible in figure 4*b*. At low light intensity the turning velocity of the mutant is much lower again, but as can be seen in figure 4*b* the final direction is towards the light source. When the mutant is surrounded by charcoal its turning velocity is lowered only slightly, but motion towards the light is again the only stable solution.

When the initial angle is larger than 90°, little light is focused on the distal side. However, some light is still focused on this side every time a cAMP wave passes through the slug, because chemotaxis towards cAMP causes the slugs' boundary to become temporarily lenticular. This light causes slow but steady turning. When the angle becomes less than 90° the tip acts as a lens all the time, so the slug turns much faster. We find that especially around 135° turning occurs very slowly. This creates the

optical impression that these angles also represent stable solutions (see, for example, figure 3*c*).

In all our simulations we used a tip consisting of oscillatory amoebae, while the rest of the slug only relayed the signal. However, the change in excitability might be much more gradual, with at least all prestalk cells in the oscillatory regime (Siegert & Weijer 1992; Dormann *et al.* 1996). Therefore we performed a number of control experiments in which all prestalk cells were oscillatory, although the tip cells were still the most excitable ones ($a_{0_t} = -0.05$). All other conditions were kept the same. This caused no qualitative differences in any of the the model behaviours: the high frequency oscillations in the tip enforced the global cAMP dynamics.

Another assumption we made is that both cAMP and NH_3 can freely diffuse in and out of the slug. For NH_3 (a volatile gas) this assumption is very reasonable, but for cAMP it is less clear what would be the correct boundary conditions. Therefore we also tested if our results depend on a specific choice of the boundary conditions. We repeated our experiments using Neumann boundaries, i.e. as if the slugs' boundary is impermeable to cAMP. We found that it does not affect the phototactic behaviour. However, the shape of the slug is less well-preserved.

4. DISCUSSION

We have described a mechanism that can explain phototaxis in *D. discoideum*, including its bidirectional mutants. Previously proposed mechanisms, such as phototaxis due to an increase in the speed of the amoebae in the illuminated region of the slug (Bonner *et al.* 1988), or bidirectionality due to actively turning towards and away from the light to correct the deviation from the preferred direction of motion (Fisher 1997), are shown to be superfluous: all the experimental data can be explained by our mechanism, which uses only light focusing, NH_3 production, modification of the cAMP wave, and chemotactic motion.

The relative contributions to the phototactic behaviour of (i) the shift in the source of the cAMP waves towards the light, and (ii) the wave shape deformation, are difficult to discriminate because these properties both emerge from the local rules and hence can never be completely separated. For example, the velocity by which the cAMP wave moves away from a certain position affects the oscillating period and therewith the source of the new cAMP wave. However, both processes contribute to the phototaxis because if the wave does not start slanted, side-effects as described for bidirectional mutants are found, whereas without differences in wave propagation the wavefront restores itself too quickly and little directed motion is created.

During the slug stage chemotactic motion towards cAMP is hard to observe. However, there are many indications that slug motion is still driven by cAMP waves (Dormann *et al.* 1998). In our model we observe that amoeboid motion is not restricted to the actual location of the cAMP wave, but is spread out over large distances due to pushing and pulling. Especially when chemotaxis is relatively weak (e.g. $\mu = 100$), a global and continuous motion is observed in which responses to

individual cAMP waves cannot be distinguished. This behaviour is very comparable with what is observed by Bonner (1998) in migrating 2D slugs. Note, however, that the behaviour is still driven by periodical cAMP waves.

In contrast to our previous model which used a linear gradient of excitability to mimic thermotaxis, the gradient in this study is dynamic and highly nonlinear. This is due to the light focusing which changes all of the time during slug motion, and also to the NH_3 dynamics. Nevertheless, the mechanism accounts for all aspects of phototactic behaviour.

According to our model, in the first instance light information is encoded using NH_3. In the previous study we assumed a similar mechanism for the thermal information. So we propose that thermotactic and phototactic information converge at a very low level, before or while converging with the NH_3 signalling. This is in agreement with Fisher (1997) who showed in a review of phototaxis research that thermotactic mutants are seldom found with unaffected phototaxis or vice versa, and thus information integration must occur at a very early stage.

We stated earlier that individual amoebae are not able to measure the direction of irradiation. Instead, the whole slug encodes differences in luminous intensity as differences in NH_3 concentration. The NH_3 changes the local excitability and hence the shape of the cAMP wave. It is the chemotaxis towards the cAMP wave which in the end induces the turning of the slug.

Fisher (1991) objects to the idea that NH_3 is the substance important for phototaxis, because is his view the NH_3 gradient in the open air would be too low to be measured, being flattened by the very high diffusion coefficient. However, the local NH_3 gradient is not measured by the amoebae either, and in our model only global concentration differences are necessary. The only gradient measured is the cAMP gradient, which by its pulsatile nature is very steep and therefore relatively easily measurable. Obviously, no chemical could ever have such a steep steady-state gradient.

Another effect NH_3 could have on the amoebae is increasing the locomotive speed; however, there is a fair amount of dispute on this subject because some studies indicate that it does (Bonner *et al.* 1986; Van Duijn & Inouye 1991) and other studies indicate that it does not (Smith *et al.* 1982; Fisher 1997). Besides, other chemicals such as O_2 could also be able to speed up the motion (Bonner *et al.* 1995). We did not include such modifications of the locomotive speed in our model, and show that locomotive differences (whether they occur or not) are not needed for phototaxis.

Neither did we include an explicit difference in chemotaxis between the cell types, although Siegert and Weijer (1992) have shown that prestalk cells move faster than prespore cells. Nevertheless, such a difference in motility emerges in our model, solely due to differential adhesion.

Fisher (1997) proposed that with phototaxis slugs always move at a certain angle to the direction of light, but that in the case of the wild type this angle would be just too small to be measurable. Instead, we find that bidirectional phototaxis occurs when NH_3 sensitivity is too high. We did not implement different mechanisms for positive and negative phototaxis, but we found that both

emerged from our model as an effect of the turning mechanism, negative phototaxis being caused by the slanted wavefront at a certain distance behind the tip. Thus we predict that it will in fact be fruitless to search for the 'direction-dependent sign reversal in slug behaviour' which makes 'slugs that travel more directly towards the light correct their error with a turn away from the light' (Fisher 1997).

Another observation that can be clarified by our model is that when slugs are formed at high cell density, bidirectional phototaxis becomes more extreme (Fisher & Williams 1981). At high cell density NH_3 concentrations will become higher, and as a result the bidirectionality will be stronger.

Besides NH_3, there is another chemical called slug turning factor (STF) which plays a role in the process of phototaxis (Fisher 1997). We did not include this substance in our model for reasons of simplicity, but the effect of STF may be similar to that of NH_3.

The multidirectional phototaxis found by Fisher *et al.* (1985) may have been caused by a very low mean turning velocity at certain angles (see, for example, figure 4*a*). With such low turning rates, a circular histogram with multiple preferred directions will be found, together with (when individual measurements are plotted) groups of data clustered close to a zero change of angle (as presented by Fisher *et al.* 1985).

Other tactical behaviours of the slug can also be interpreted in terms of the same mechanism as that presented in this paper. First, Bonner *et al.* (1985) observed that migrating slugs tend to travel towards the acid side of a pH gradient. This so-called acidotaxis, which is weak compared to phototaxis or thermotaxis, is most probably caused by a change in the NH_3/NH_4^+ ratio (Schindler & Sussman 1979; Bonner *et al.* 1985, 1986). A low pH value decreases this ratio; hence a pH gradient will coincide with an NH_3 gradient. If NH_3, and not NH_4^+, is the active substance able to decrease excitability, then acidotaxis becomes directly comparable with the negative chemotaxis away from NH_3 as described earlier. Second, Haser & Häder (1992) found rheotaxis: slugs migrate downwind in an air stream. This also fits into our model because the NH_3 concentration will be lower on the proximal side. The same reasoning also holds for the observed motion towards a piece of charcoal provided it is not saturated with NH_3. Finally, migrating slugs repel each other (Kosugi & Inouye 1989), rising fruiting bodies orientate away from each other (Bonner & Dodd 1962) and this orientation is affected by NH_3 (Feit & Sollitto 1987). Once again, higher NH_3 concentrations between the slugs or fruiting bodies, produced by the slugs themselves, could account for this behaviour.

Although all behaviours can be explained by one basic mechanism, they all work together to ensure that the final fruiting takes place in a favourable environment: they let the slugs move towards the soil surface and fruit at regular spatial intervals.

5. CONCLUSIONS AND PREDICTIONS

Our study shows that a whole set of previously described phototactic behaviours can be explained solely with differences in NH_3 concentration, which are caused

by differences in luminous intensity, and which cause alterations of the excitability. The model predicts that no 3D properties are needed for phototactic behaviour. The behaviour of the simulated 2D slugs is strikingly similar to that of the experimental 2D slugs of Bonner (1998), and both resemble the behaviour of 3D slugs closely. We predict that phototaxis by wild type and mutants can also be observed in experimental 2D slugs. The motion of individual cells can be followed in the simulations and also in the above mentioned experiments. Our simulations make strong predictions about the directions of motion during phototaxis of individual amoebae of the wild type and mutants.

The specific form of the tip, as normally observed in migrating slugs, is neither found in our simulations, nor in the experiments of Bonner (1998). Indeed, Siegert & Weijer (1992) showed that the tip is organized by 3D scroll waves. Hence we predict that the specific form of the tip is a 3D property, which cannot occur in 2D slugs. However, we have demonstrated that this is not essential for the behaviour studied here. We therefore predict that the thin elongated tip is less of an organizing centre than has been previously proposed.

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