Seeing around corners: Cells solve mazes and respond at a distance using attractant breakdown.

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Abstract:

During development and metastasis, cells can migrate large distances through complex environments, guided by chemoattractants. Simple gradients between a source and sink cannot guide cells over such long ranges. We describe how cells can navigate accurately through tortuous, branched paths using gradients they create by locally degrading attractant. We found that these self-generated gradients enabled single cells to make accurate choices at branches, essentially allowing them to see around corners and solve complex mazes with remarkable efficiency. Accuracy was controlled by attractant diffusivity, cell speed, and path complexity. Inappropriate combinations of these parameters actively misdirected cells in mathematically predictable ways. We propose that self-generated gradients impact many long-range migratory processes, including inflammation and germ cell migration, where path complexity and length require them.

One Sentence Summary:

Cells navigate through complex environments and solve mazes by creating their own
30 chemotactic gradients.

Main Text:

Cells migrating in embryogenesis (*1–3*), immune responses (*4*, *5*) and neural pathfinding (*6*, *7*) steer using chemotaxis, migrating up gradients of attractants such as chemokines and netrins. Simple chemotaxis, in which gradients are established between a localised attractant source and an external sink, only provides short-range guidance (*8*). It becomes inefficient at distances above 500µm (*9*) and can only use a narrow range of attractant concentrations. These restrictions have confounded our understanding of how chemotaxis drives longer-range phenomena such as neural crest migration (*10*) and cancer metastasis (*11*). However, when cell groups locally break down an attractant found throughout the surroundings, they create their own local, dynamic gradients (*1*, *12–14*), which typically direct them away from areas with a high density of cells, promoting metastasis (*15*). These self-generated gradients work over arbitrarily long distances, and work equally robustly with a wide range of attractant concentrations (*16*). This paper tests their role in resolving complex paths, as for example a cell migrating through an embryo would follow. Cells using self-generated gradients are able to make accurate choices about paths they have not yet encountered. This enables them to solve complex mazes, even when the initial environment is homogeneous and the correct destination is distant. Computational models, combined with live cells in microfluidic devices (*17*), reveal how the accuracy of decisions in complex environments is determined by the complexity and lengths of the paths, and the speeds of the cells. This mechanism explains how cells can traverse longer distances than seems possible given receptor parameters, and can interpret environmental features in a way that would be impossible with simple attractant sources.

Self-generated gradients are efficient.

Chemotactic cells detect attractant gradients by comparing receptor occupancy at different places. Cells can resolve 1% differences (*18*) between the occupancy at their fronts and rears, but this is only enough to navigate short distances – beyond 0.5-1mm, gradients contain zones that are either too shallow or too saturating to cause a 1% occupancy difference, and thus be detectable (*9*). However, cells can make sharp, local gradients by breaking down attractants. The resulting dynamic, self-generated gradients are usually impossible to measure directly, so are

best studied using computational models, verified experimentally using living cells. Fig. 1A & Movie S1 show modelled cells responding to a passive 1mm gradient or to a self-generated gradient in which the attractant is broken down by a cell-surface enzyme. Secreted enzymes give similar results (*9*). Passive gradients are either too shallow or, if steeper, rapidly saturate the receptors. Cells therefore always steer poorly in simple 1mm gradients. In comparison, the self-generated gradient gives robust chemotaxis throughout, because the gradient is always sharp and locally-produced, with a non-saturating attractant concentration around the cells (Fig. 1A & Movie S1). Real cells behaved the way the model predicts (Fig. 1B, Movie S1).

Self-generated gradients allow cells to make long-range route decisions

Chemotaxis studies often ignore diffusion, because they consider the steady state of imposed, linear gradients (*19*, *20*). For dynamic gradients, such as self-generated gradients, it is a key determinant. Feedback between cells depleting attractant, fresh attractant diffusing towards them, and their migration in the resulting gradients can yield counterintuitive results and must be analysed in detail. We therefore modelled the way cells make decisions at junctions (Fig. 1C-E). With the simple case of two equivalent routes to an attractant reservoir (Fig. 1C), self-generated and static gradients already gave different behaviour. In the static gradient each cell chose a route randomly. The self-generated gradient, on the other hand, robustly directed equal numbers of cells into each branch. Stochastic variations were balanced out – branches containing more cells evolved shallower attractant gradients, so newly-arriving cells were directed into the other branch (shown quantitatively in Fig. 1F). If one of the branches was closed off from the reservoir (Fig. 1D), only a small number of cells entered the closed branch, because they rapidly depleted the attractant and prevented further recruitment.

When the closed branch was shorter (Fig. 1E), an unexpected behaviour emerged from the modelling. The migrating cells cleared the attractant from the short branch by diffusion, before they reached the junction and decided a direction. Cells thus sensed the space ahead of themselves at a distance by breaking down a diffusible chemoattractant; they can sense a closed branch without entering it, and make accurate decisions about pathways ahead they have not visited.

The effect depended strongly on the length of the dead end. Fewer than 10% of cells commited to dead ends <250µm (Fig. 1G). 40% commited at >600µm, because the time taken for attractant to diffuse out of the dead end increased quadratically with its length, emphasizing the importance of diffusion.

To understand how cells sense closed paths without visiting them, we modelled cells following self-generated gradients through complex routes, analogous to physiological problems like finding a path from a tumour into a blood vessel. A single "correct" path connects each starting well to a reservoir of attractant. Parameters were taken from measurements of *Dictyostelium* cells (*21*, *22*), which chemotax towards 3',5'- cyclic adenosine monophosphate (cAMP) while breaking it down using a cell-surface phosphodiesterase (*23*). We verified the models' predictions by fabricating identical microfluidic mazes in polydimethylsiloxane (PDMS; Fig. S2). All designs worked well, despite starting with uniform chemoattractant so there were initially no directional cues. As seen below, the simulations accurately predicted the behaviour of real cells (Figs 2,3,5,6).

Self-generated gradients allow cells to navigate mazes

Cells can navigate environments of surprising length and complexity by combining the long range of self-generated chemotaxis with the ability to detect dead ends ahead (Fig. 2). To ensure that the *Dictyostelium* were not convolving the results by generating their own cAMP signals, we generated a new adenylyl cyclase (*aca*A) mutant in a wild-type (NC4) parent, which is healthier and migrates better than more widely used axenic strains. These cells recapitulated the models with remarkable accuracy (Movies S2-4; compare Figs 2A/B, 2D/E & 2G/H). We confirmed that the gradients were self-generated using a nondegradable ligand. Cells made almost no progress when the breakdown-resistant cAMP analogue Sp-cAMPS (*24*) was used instead of cAMP (Fig. S1, lower panels). This, together with the wide and tall (40µm x 25µm) maze channels, also argues against strong hydraulic effects (*Dictyostelium* cells are <10µm wide and about 5µm tall).

Self-generated gradients thus allowed cells to navigate mazes that were too long and complex for simple imposed gradients to be readable.

Accuracy of decisions is controlled by length and complexity of paths

We compared cells' decisions at junctions in three different maze designs (Fig. 2A, D & G). Each had the same correct path, but "simple" mazes had three short, dead ends (Fig. 2A-C), "short" mazes had dead ends half the length of their live ends (Fig. 2D-F), and "long" mazes had symmetrical live and dead ends, except the connection to the attractant reservoir (Fig. 2G-I). We verified the model's predictions using *Dictyostelium* cells and mouse pancreatic cancer cells that self-generate gradients of lysophosphatidic acid (LPA) in serum (*25*).

The model predicted that most cells avoid dead ends in the simple mazes (Fig. 2A, Movie S2). Real cells behaved the same way, with a small group migrating accurately through the maze, and few cells deviating into the dead ends (Fig. 2B-C, Movie S3-4). Decisions were poorer in short mazes (Fig. 2D-F), and many cells chose the dead ends in long mazes (Fig. 2G-I).

To measure the dynamic fidelity of decisions, we recorded the cumulative number of cells committing to the dead and live ends, initially focussing on the first, hardest decision in each maze (Fig. 3A, B). This confirmed that longer dead ends lead to poorer decision fidelity - cells performed consistently better in simple vs. long mazes, and better in general in short vs. long mazes (Fig. 3C-E; blue line shows numbers of cells choosing the correct path, and red the number committing to the dead end). Long mazes were significantly less biased over the first hour than either other design (Fig. 3F; one way ANOVA and Tukey's test, α=0.05).

Responses for longer mazes were biphasic – initially there was little difference between the numbers of cells choosing correctly and incorrectly, but as cells depleted the attractant in the dead ends their decisions became more accurate (Fig. 3C, D). Pancreatic cancer cells (in homogeneous medium containing 10% serum; Fig. 3E, Movie S4) confirmed this is a general feature of self-generated gradients; their decision-making showed the same trend as the *Dictyostelium* cells, though they moved much slower, with increasingly accurate decisions being made as the dead ends became shorter. In both simulations and experiments, later decisions were more accurate (Fig. 3G).

Cell speed and attractant diffusivity

Modelling and experimental approaches were supplemented with a mathematical analysis, which considered the effects of live and dead end lengths on a decision at a T junction (described in detail in SI section 3.1). As real cells are not static when they decide a direction at junctions, we developed a mathematical mapping connecting the cell speed to a static waiting time; this was successfully validated by comparing it to simulations. The analysis yielded three key predictions, shown in Fig. 4A. First, shorter dead ends gave more accurate decisions; second, shorter live ends also gave more accurate decisions; and third, decisions were more accurate if cells took longer to make them.

We had already observed the first prediction experimentally, strengthening our trust in the others. The second happened both because equilibrium was quickly reached, and because the resulting well-to-junction gradient was steeper. The third was due to cells having longer to clear attractant diffusing out of dead ends before deciding which path to take. This explains the cells' greater accuracy in the second and third decisions in the mazes (for example Fig. 3G, Movies S2-3). The same pattern was observed when altering cell speed in simulations, as slower cells effectively had longer to make decisions (Fig. 4B; note similarity to 4A).

These findings raised an apparent contradiction. We predicted that slower cells would make better decisions than faster ones, yet *Dictyostelium*, which solve mazes in two hours, performed similarly to cancer cells that take roughly two days. The attractants, cAMP (*26*) and LPA (*15*) respectively, have similar molecular masses, so we had expected them to have similar diffusivities. However, lipids are often carried by proteins such as albumin, slowing their effective diffusion. We predicted (Fig. 4C) the relationship between the diffusivities of cAMP and LPA that would lead to equal decision fidelity, and then performed a photobleaching assay on fluorescently labelled LPA and cAMP (Fig. 4D-E). The effective diffusivity of LPA was strongly reduced (to about $1/10^{th}$), as expected, with the relationship between the molecules similar to that predicted mathematically (about $1/20^{th}$, SI Section 3.2).

This result emphasized the effect of diffusion on maze accuracy. We therefore simulated the three maze designs using attractants with a range of diffusivities. The mathematical analysis

predicted that high diffusivity would yield excellent decisions, and that fidelity would decrease with diffusivity until decisions were made with no information, so 50% of cells head in each direction. The models followed this prediction, with one surprising exception - cells in the short maze were predicted to do worse than 50:50 for diffusivities around 1/10th that of a small molecule like LPA or cAMP (Fig. 4F-G). This effect was not seen in long mazes, implying that shorter dead ends caused greater misdirection than longer ones. On detailed inspection, two or more dead-end channels in the short maze were close enough to the junction to supply diffusing attractant molecules, whereas in the live end, molecules travelling a similar distance only came from a single channel. The quadratic relationship between diffusive flux and distance meant that brancjes further up the live path had a minimal effect.

Complex topologies drive cells into incorrect decisions

This leads to a surprising prediction – a dead end can be more attractive than a live one, if it is shorter and branches or widens. This can create chemotactic mirages, which lead cells away from the source of attractant.

We built new mazes to test whether mirages were practical under normal conditions. Each connected the cell well and large attractant reservoir with a path of identical length (around 800µm). After a variable approach, cells encountered a T junction, at which they could either steer toward the large reservoir of attractant or down a dead end, again of variable length, towards a smaller attractant well (Fig. 5A-C). We created and tested 16 designs in total, with four approach lengths (150, 300, 450 & 600µm) and four dead end lengths (150,300,450 & 600µm).

We expected shorter approach lengths to yield more severe mirages for two reasons. First, a shorter approach time gives cells less time to clear attractant from the dead end. Second, the attractant well down the live path would be further away, and would therefore signal more weakly. We also expected short dead ends to generate stronger mirages, as short-range diffusion would supply more attractant molecules from the side well. Finally, if the path to the live end is shorter than the path to the dead end, no mirage should form at all.

In simulations and experiments, short-approach, short-dead-end mazes produced a profound mirage, steering cells toward the trap (Fig. 5A, D, top panels). In intermediate designs, signals from the true reservoir were strong enough to cause some cells to reverse their initial, incorrect decision (Fig. 5 B, D, middle panels, & Movie S5). For long approaches and dead ends, the stronger signal of the large reservoir dominates decision-making, so there is no mirage (Fig. 5C, D, bottom panels). Interestingly, simulations of these designs made an additional prediction- as these easy-to-avoid mirages move the trap a large distance away from the cell well, flux through the entrance of the maze was reduced, so fewer cells were recruited (Fig. 5D- compare top and bottom panels). We scored each design using the decision bias through the first hour - the relationship between the lengths and decision-making holds true across all conditions and is accurately predicted by the model (Fig. 5E). Interestingly, the dead-end mazes confirm that most phosphodiesterase is bound to the cell surface; models where the enzyme diffuses reproduce the data much less well (Fig. S7)

These results emphasise a key message for self-generated chemotaxis: the key determinant is attractant flux, not quantity. Longer dead ends contain more attractant, but signal more weakly. This is obviously relevant to migration in vivo; cancer cell metastasis, for example, favours tracks with low resistance (*27*), which allow greater flux of attractants.

Targeting specific outcomes in topology design.

As a final test, we made two pairs of closely-related mazes. For each pair, the paths to the source and dead-end space are similar, but one was designed to be “easy” (models predict accurate decisions) and one “hard” (Movies S6-7). Easy mazes had short, weakly branched dead-ends. Hard mazes had long, highly branched dead ends, with branching beginning near the entry to maximise the mirage effect (Fig. 6).

We scored the progress of cells through the first pair of mazes (the labyrinth designs, Fig. 6A-C), counting how many passed a checkpoint after each major decision (Fig. 6A, yellow, blue & purple spots marking the checkpoints). Cell behaviour in these designs was strikingly similar to that predicted by the simulations (Fig. 6B&C show the first checkpoint for the easy and hard designs, respectively). Cells scored extremely divergently in the two designs, despite their

superficial similarity (Fig. 6D). A second pair of designs (the trident designs, Fig. 6E-G) yielded very similar results (Fig. 6F-H). In both design pairs, large parts of the easy mazes were not visited when compared with their hard counterpart (Fig. 6I&J). Thus understanding the
235 principles of chemotactic mazes can accurately inform how real cells respond to complex environments.

Discussion

This work has clear and wide-ranging implications for biology in general. We find that the details of cells' environments strikingly affect their ability to steer accurately, and allow them to steer using information it seems impossible for them to have obtained. Changes that seem minor make a substantial – and predictable - difference to the accuracy and eventual destination of the cells . By matching our experimental results to mathematical and computational methods, we ensure that our findings are general, applying to any system employing an attractant that can be degraded by the cells that respond. This mechanism differs entirely from that used by *Physarum polycephalum* to solve mazes (*28*), which relies on the plasmodium migrating down all branches simultaneously before pruning useless paths (*29*).

Many situations where chemotaxis occurs in vivo – neutrophils extravasating to an infection in tissue (*30*), for example, or germ cells migrating through an embryo (*31*) – have equivalently complex paths. Similarly, attractant degradation is widespread, with examples known in immunity (*32*), development (*31*) and cancer (*33*). Chemotactic cells have a variety of mechanisms for depleting attractants, including receptor-ligand endocytosis (*34*), decoy receptors (*1*, *12*, *35*), and cell-surface enzymes that degrade attractants (*33*, *36*). Ligand breakdown is rarely considered when interpreting spatial patterns of chemotaxis data - its effects can be complex, counterintuitive and difficult to measure - but our results show it needs to be analysed and understood. Additionally, many cells create attractants as well as degrading them, and additional attractants may influence behaviour independently. All of these processes complicate the picture, and all emphasise the pivotal role in the future of biology of computational modelling, combined with verification using experiments and modern microscopical techniques.

We have described decisions as better or worse, but these loaded terms need not apply to an in vivo context. Our aim is to understand how complex topology draws cells to places that intuition would not predict. The chemotactic mirage, in which expanding and branching topologies demonstrate more powerful chemoattraction than an actual source of chemoattractant, is particularly counter-intuitive. This could be crucial to understanding migratory behaviour in complex in vivo environments (*11*), for example neutrophil extravasation into tissues, migration

of melanoblasts through the embryonic dermis, or metastasis of glioblastoma through white matter tracts of the brain (*37*). Overall, the detailed interaction between cells' breakdown of chemoattractants and the geometry of the paths they chemotax along predicts fundamental behaviours in complex environments, and remains an under-studied area of biology with insights into basic physiology.

Methods

Cell Lines: *aca- D. discoideum* were generated by homologous recombination in an NC4 parental background using resuspended bacteria, according to ref (*38*), and were grown non-axenically on bacterial lawns. To sensitise cells to attractant, they were collected at 1.5-3x10^7 cells/ml in development buffer (DB) and shaken for 1h, then pulsed at 6min intervals for 3.5-4h with 300nM cAMP. Cells were then pelleted and resuspended in maze medium (DB + 2.5µM cAMP + 0.05% BSA), and inoculated into the cell well at high density (~90% confluency). The cancer line used was KPC model murine pancreatic cancer (-kras -p53). Cells were cultured in DMEM+20%FCS, then trypsinised, resuspended in DMEM+10% fresh FCS and placed in the cell well of the maze. In these experiments, mazes were filled with DMEM+10% fresh FCS.

Basic chemotaxis experiments: *D. discoideum* cells were harvested, collected at 1.5-3x10^7 cells/ml in development buffer (DB) and shaken for 1.5h. Cells were then pulsed with 300nM cAMP for 4.5h at 6min intervals, bringing them into an aggregation competent state.

For fig.1 an Insall chamber was used to create a stable attractant gradient in a viewing bridge between two connected reservoirs of attractant. For an imposed gradient, the inner attractant reservoir was filled with DB containing 1µM Sp-cAMPS and the outer reservoir with attractant-free DB. For a self-generated gradient, both the inner and outer reservoir were filled with 10µM cAMP in DB. Cells were mixed into the outer well medium at a density of 2.5x10^6/ml.

Maze design and fabrication: Design schematics for mazes are given in the SI. Microfluidic mazes were fabricated in polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, US) using standard soft lithography techniques. Briefly, silicon masters were produced using SU8 3005 photoresist (3000 series, MicroChem, US) on silicon wafers following the manufacturer's protocol to achieve a final resist thickness of 25 µm. The resist was exposed through a photomask (JD Photo-Tools, UK) to collimated UV light and was developed in MicroPosit EC solvent (Rohm and Haas, US). To prevent PDMS adhesion to the silicon master, this was salinized by vapour deposition of 1H,1H,2H,2H perfluorooctyl-trichlorosilane for 1h. PDMS was poured onto the silicon master at a 10:1 ratio of base to curing agent, degassed in a vacuum desiccator chamber and cured at 70°C for at least 3 hours. PDMS devices were then peeled from

the mould, cut to the desired size and 2mm holes were punched to obtain inlet and outlet ports. PDMS devices were then cleaned and irreversibly bonded to glass-bottom petri dishes (manufacturer) using oxygen plasma.

Maze use: Mazes were filled uniformly with medium by filling all inlet ports with the medium of choice (typically ~6µl per well) and then placing into a vacuum desiccator for around 20min, degassing the PDMS. When the vacuum is released, the pressure difference draws medium into all parts of the maze, including dead ends, although this functions best if additional medium is pipetted up and down in each well, as this dislodges residual gas bubbles. Mazes used for cancer cells were pre-filled with 0.05% BSA in sterile, deionised water in order to block the PDMS and prevent any attractants from adhering. The pre-fill was dried out by first draining the wells thoroughly with a pipette, then placing in a tissue culture hood for ~2h. As soon as a maze was observed to be dry, it was re-filled with experimental medium (which, for cancer cells was Dulbecco's Modified Eagle Medium (DMEM)+10% FCS, freshly added).

Simulations: Simulations were written in Java. Diffusion in a complex environment was simulated using the semi-implicit DuFort-Frankel method. Agent-based model cells then made decisions using a persistent, biased random walk. The persistent, random element comes from drawing a new direction at each step from wrapped-normal distribution centred on the current direction of motion. The attractant gradient direction is estimated from grid points that overlap the cell (all those grid-points within 6µm of the cell centroid) and this is used to generate a bias vector. The bias vector is added to the persistent, random vector to choose a final direction of motion, and the cell moves in this direction at its current speed unless it collides with a wall- in which case, its movement distance is reduced.

Cells degrade attractant at a rate r determined by Michaelis Menten kinetics, *i.e.*

$$r = v_{max}\frac{c}{c+k_m},$$

with attractant removed evenly from all grid points overlapped by the cell.

Analysis: All decisions in mazes were binary, with cells committing to a live or a dead end. *D. discoideum* cells were counted as having committed once the whole cell body passed out of the junction into one or the other channel. Cancer cells, which are much larger, were counted as having committed once the nucleus left the junction. Decision reversals were tracked, with a cell

re-entering the junction lowering the score of its channel. In order to account for some stochastic variation from random movement, all mazes were timed from the arrival of the second cell.

All maze figures involve comparisons between designs. In all cases, these different designs were tested against one another with the same cells on the same day.

Long, short and simple mazes in Fig. 2 & 3: N=3, with 14, 14 and 12 technical replicates used for these mazes respectively.

Mirage mazes in Fig. 5: N=4, with 9-12 technical replicates performed in total for each design.

Easy and hard mazes in Fig. 6A: N=3, with 11 technical replicates of each in total.

Easy and hard mazes in Fig. 6C: N=3, with 12 technical replicates of each in total.

References and Notes:

1. E. Dona *et al.*, Directional tissue migration through a self-generated chemokine gradient. *Nature* **503**, 285 (2013).
2. M. Chuai, C. J. Weijer, Regulation of cell migration during chick gastrulation. *Current opinion in genetics & development* **19**, 343 (2009).
3. X. Yang, D. Dormann, A. E. Münsterberg, C. J. Weijer, Cell movement patterns during gastrulation in the chick are controlled by positive and negative chemotaxis mediated by FGF4 and FGF8. *Developmental cell* **3**, 425 (2002).
4. B. A. David, P. Kubes, Exploring the complex role of chemokines and chemoattractants in vivo on leukocyte dynamics. *Immunol Rev* **289**, 9 (2019).
5. I. R. Evans, W. Wood, Drosophila blood cell chemotaxis. *Curr Opin Cell Biol* **30**, 1 (2014).
6. C. E. Adler, R. D. Fetter, C. I. Bargmann, UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. *Nat Neurosci* **9**, 511 (2006).
7. T. F. Sloan, M. A. Qasaimeh, D. Juncker, P. T. Yam, F. Charron, Integration of shallow gradients of Shh and Netrin-1 guides commissural axons. *PLoS Biol* **13**, e1002119 (2015).
8. L. Tweedy, O. Susanto, R. H. Insall, Self-generated chemotactic gradients-cells steering themselves. *Curr Opin Cell Biol* **42**, 46 (2016).
9. L. Tweedy, D. A. Knecht, G. M. Mackay, R. H. Insall, Self-Generated Chemoattractant Gradients: Attractant Depletion Extends the Range and Robustness of Chemotaxis. *PLoS Biol* **14**, e1002404 (2016).
10. R. McLennan, J. M. Teddy, J. C. Kasemeier-Kulesa, M. H. Romine, P. M. Kulesa, Vascular endothelial growth factor (VEGF) regulates cranial neural crest migration in vivo. *Dev Biol* **339**, 114 (2010).
11. E. T. Roussos, J. S. Condeelis, A. Patsialou, Chemotaxis in cancer. *Nat Rev Cancer* **11**, 573 (2011).
12. G. Venkiteswaran *et al.*, Generation and Dynamics of an Endogenous, Self-Generated Signaling Gradient across a Migrating Tissue. *Cell* **155**, 674 (2013).
13. S. R. Schwab, J. G. Cyster, Finding a way out: lymphocyte egress from lymphoid organs. *Nat Immunol* **8**, 1295 (2007).
14. C. Scherber *et al.*, Epithelial cell guidance by self-generated EGF gradients. *Integr Biol (Camb)* **4**, 259 (2012).
15. A. J. Muinonen-Martin *et al.*, Melanoma Cells Break Down LPA to Establish Local Gradients That Drive Chemotactic Dispersal. *PLoS Biol* **12**, e1001966 (2014).
16. L. Tweedy, R. H. Insall, Self-Generated Gradients Yield Exceptionally Robust Steering Cues. *Front Cell Dev Biol* **8**, 133 (2020).
17. D. Irimia, Microfluidic Technologies for Temporal Perturbations of Chemotaxis. *Annu Rev Biomed Eng* **12**, 259 (2010).
18. S. H. Zigmond, Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes. *Nature* **249**, 450 (1974).
19. S. H. Zigmond, J. G. Hirsch, Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. *J Exp Med* **137**, 387 (1973).

20. D. Zicha, G. A. Dunn, A. F. Brown, A new direct-viewing chemotaxis chamber. *J Cell Sci* **99**, 769 (1991).
21. P. J. Van Haastert, Binding of cAMP and adenosine derivatives to Dictyostelium discoideum cells. Relationships of binding, chemotactic, and antagonistic activities. *J Biol Chem* **258**, 9643 (1983).
22. L. Wu, J. Franke, R. L. Blanton, G. J. Podgorski, R. H. Kessin, The phosphodiesterase secreted by prestalk cells is necessary for Dictyostelium morphogenesis. *Dev Biol* **167**, 1 (1995).
23. G. Gerisch, D. Hulser, D. Malchow, U. Wick, Cell communication by periodic cyclic-AMP pulses. *Philos Trans R Soc Lond B Biol Sci* **272**, 181 (1975).
24. C. Rossier, G. Gerisch, D. Malchow, Action of a slowly hydrolysable cyclic AMP analogue on developing cells of Dictyostelium discoideum. *J Cell Sci* **35**, 321 (1979).
25. A. Juin *et al.*, N-WASP Control of LPAR1 Trafficking Establishes Response to Self-Generated LPA Gradients to Promote Pancreatic Cancer Cell Metastasis. *Dev Cell* **51**, 431 (2019).
26. Y. Artemenko, T. J. Lampert, P. N. Devreotes, Moving towards a paradigm: common mechanisms of chemotactic signaling in Dictyostelium and mammalian leukocytes. *Cell Mol Life Sci* **71**, 3711 (2014).
27. P. G. Gritsenko, O. Ilina, P. Friedl, Interstitial guidance of cancer invasion. *The Journal of pathology* (2012).
28. T. Nakagaki, H. Yamada, A. Tóth, Maze-solving by an amoeboid organism. *Nature* **407**, 470 (2000).
29. T. Nakagaki, H. Yamada, A. Tóth, Path finding by tube morphogenesis in an amoeboid organism. *Biophys Chem* **92**, 47 (2001).
30. E. Kolaczkowska, P. Kubes, Neutrophil recruitment and function in health and inflammation. *Nature reviews immunology* **13**, 159 (2013).
31. A. D. Renault, P. S. Kunwar, R. Lehmann, Lipid phosphate phosphatase activity regulates dispersal and bilateral sorting of embryonic germ cells in Drosophila. *Development* **137**, 1815 (2010).
32. B. Breart *et al.*, Lipid phosphate phosphatase 3 enables efficient thymic egress. *J Exp Med* **208**, 1267 (2011).
33. O. Susanto *et al.*, LPP3 mediates self-generation of chemotactic LPA gradients by melanoma cells. *J Cell Sci* **130**, 3455 (2017).
34. M. A. Puthenveedu *et al.*, Sequence-dependent sorting of recycling proteins by actin-stabilized endosomal microdomains. *Cell* **143**, 761 (2010).
35. A. Rot *et al.*, Cell-autonomous regulation of neutrophil migration by the D6 chemokine decoy receptor. *J Immunol* **190**, 6450 (2013).
36. G. L. Garcia, E. C. Rericha, C. D. Heger, P. K. Goldsmith, C. A. Parent, The Group Migration of Dictyostelium Cells Is Regulated by Extracellular Chemoattractant Degradation. *Mol Biol Cell* **20**, 3295 (2009).
37. C. Denier, A. F. Carpentier, K. Mokhtari, J. Y. Delattre, Cerebral glioblastomas and systemic metastases. *Revue neurologique* **157**, 1531 (2001).
38. P. Paschke *et al.*, Rapid and efficient genetic engineering of both wild type and axenic strains of Dictyostelium discoideum. *PLoS One* **13**, e0196809 (2018).

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Competing interests:

Authors declare no competing interests

Data and materials availability:

All data is available in the main text or the supplementary materials.

Code availability:

Source code is available on github at https://github.com/ltweedy/MazeNavigation.

Supplementary Materials:

Materials and Methods

Movies S1-S7

SI Submission (mathematical treatments)

Fig. 1: Self generated gradients allow cells to explore remote features.

(A) Comparison of imposed and self-generated gradients guiding cells across 1mm to a full attractant well. **(B)** Experimental verification of (A) using *Dictyostelium discoideum*. The imposed gradient uses the non-degradable attractant Sp-cAMPS. The self-generated gradient uses uniform cAMP. Cells following the imposed gradient perform worse, especially after the halfway point. Bar 50µm. See Movie S1.

(C-E) Simulated navigation past a junction. In (C) both branches are identical and connect to an attractant reservoir. Each recruits the same number of cells. In (D) one branch is a dead end. Some cells do still commit due to residual attractant in the channel. In (E), the dead end is much shorter, and is almost entirely free of attractant as the cells reach the junction. **(F)** Number of cells selecting the top channel through repeated simulations of (C). Concentration is tuned so an average of 24 cells commit. The self-generated gradient has a smaller standard deviation than a random choice, revealing active sorting. **(G)** The fraction of cells committing to a dead end as a function of its length. Few cells commit to short dead ends, but there is an apparently linear increase from 250µm-650µm. Above this, the fraction plateaus at about 0.4.

Fig. 2: Real cells can solve mazes.

(A) Simulation of cells navigating the maze with short dead ends, at time-points past the first, second and third decisions. In this design, cells are predicted to almost always commit to the correct path to lead them to the attractant well. **(B)** *Dictyostelium* cells migrating through the same maze design, initially filled uniformly with the attractant cAMP. **(C)** Pancreatic cancer cells in the same maze design, with an initial background of 10% FCS. **(D-F)** Simulations of the short-branched maze (D), compared with *Dictyostelium* (E) and pancreatic cancer (F) cells navigating the same design. **(G-I)** Simulations of the long-branched maze design (G), compared with *Dictyostelium* (H) and pancreatic cancer (I) cells. See Movies S2 (A,D,G) , S3 (B,E,H) & S4 (C,F,I). Device width 850µm; channel width 40µm; channel height 25µm.

Fig. 3: More distant and complex dead ends are harder to resolve.

(A) Simulations of the long-, short-, and simple-branched mazes as the cells reach the junction between the live path and the first dead end. **(B)** The same simulations at a later time. Cell colour has been altered for those that have committed to the live end (deep blue) or dead end (red). Uncommitted cells are shown in the original grey blue. **(C-E)** Running totals of cells committed to the live end (blue) and dead end (red), as pictured in (B). Results are shown for simulations (C), *Dictyostelium* (D) and pancreatic cancer experiments (E), with t=0 when cells first reach the entrance to the maze. Light blue shading highlights the difference between these values. **(F)** Decision fidelity scores for *Dictyostelium* cells at the first junction of each maze. The simple- and short-branched mazes both differ significantly in their fidelity to the long-branched maze (one-way ANOVA, α=0.05). **(G)** Overall decision fidelity scores for the simulations compared with their experimental counterparts. Decision fidelity is $\langle t - f \rangle / \langle t + f \rangle$ over a 1hr window, where t and f are the number currently committed to the correct and incorrect paths. Later decisions generally have higher fidelity.

Fig. 4: Slower cells and faster diffusion lead to more accurate decisions.

(A) Mathematical model of decision fidelity at a T junction. Each panel shows a snapshot after a different waiting time at the junction before making decision. Lengths of the live and dead ends on x and y axes, respectively. **(B)** Decisions made by simulated cells moving at different speeds, and therefore taking different times to reach the junction so as to correspond with the panels above. **(C)** To correct for cell movement sharpening the gradient, we create a mapping from the solvable static model to an adjusted model. The static and adjusted models are shown for three cell speeds against the true gradient seen in the simulations. **(D)** Photobleaching of 8-fluo-cAMP and TopFluor Lyso PA. **(E)** Recovery curves & fits for the photobleached areas suggest that the effective diffusivity of LPA is ~$1/10^{th}$ that of cAMP. Bar 50µm. **(F)** Expected decision fidelities for the short-branched maze across time, simulated over a variety of attractant diffusivities. Notably, a phase change in behaviour occurs for lower diffusivities, in which a majority of cells choose the dead end. This does not happen in the long-branched maze, revealing that, in branching topologies, shorter dead ends may in fact lead to worse decisions. **(G)** Snapshots of three simulations at the three numbered points in (F).

Fig. 5: Cell decisions depend on the rate of attractant transport.

(A-C) Using 4 dead-end lengths and 4 junction approach lengths (labelled in (B)), we generate 16 mazes in total with varying predictions of cell behaviour. We show real cells (left) and simulation predictions (right) for 3 of the 16 designs. (A) shows short approach length and dead-end length (both 150µm). (B) shows a 150µm dead end and a 450µm approach, with cells faring better than in (A). (C) shows an approach length and a dead-end length of 600µm. In this case, cells overwhelmingly steer correctly toward the large reservoir. Dynamics of (A-C) can be seen in Movie S5. Distance between reservoirs 800µm. **(D)** Total number of cells committing to the live- and dead-ends on average for the designs shown in A-C. Blue filling shows a positive bias (favouring the live end), while red filling shows a negative bias (favouring the dead-end). For a short approach and a short dead end, real cells have a strongly negative bias which is not

overcome within the hour-long observation (top-left). This was predicted by simulation (top-right). An intermediate approach-length and a short dead end causes a brief chemotactic mirage, but the dead-end is short enough that the misdirected cells then receive attractant flux from the large reservoir and rectify their mistake (middle left). This again is predicted (middle-right). A long approach and long dead-end (bottom-left) has two features. Firstly, the bias is consistently positive and no mirage forms. Secondly, this design recruits fewer cells overall. Both features were predicted by the simulations. (**E**) Average bias score over a 45min observation for all 16 designs. The general trend of short approach and dead ends both causing negative bias, predicted by the model (right), is seen clearly in the experimental data (left).

Fig. 6: Deliberate misdirection of live cells.

(**A)** Designs for easy and hard labyrinth mazes. Though both have the sa me amount of dead-end space, the hard labyrinth is designed with fewer, longer, and more branched dead ends. We note that the easy labyrinth is harder to solve visually. Checkpoints are marked (coloured dots). These are used to score the navigational success of cells. Devices 800µm wide. (**B-C**) Example images of cells passing the first checkpoint in both labyrinths, alongside the behaviour predicted by simulations. See Movie S6. **(D)** Number of *Dictyostelium* cells passing each checkpoint in (A) as a fraction of maze entrants. The checkpoint colour tag is shown above each value. (**E**) Designs for easy (left) and hard (right) trident mazes along similar principles to (A). Devices 810µm wide (**F-H**) Same as (B-D) for the trident designs. See Movie S7. Pairwise *t*-testing the final decision yields $p<0.05$ for both design pairs. **(I-J)** Cell images taken at 5min intervals are colour-coded by time and superimposed on the unmoving, median image of the mazes for all four designs, showing the path taken by cells.

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