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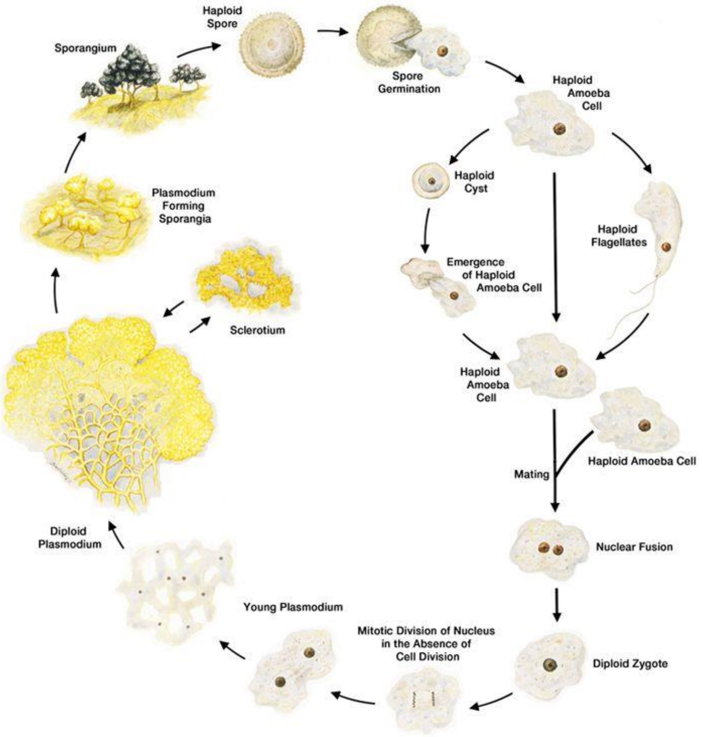
Background on *Physarum polycephalum*

Many of the processes used by *Physarum* are similar to those found in more complex organisms. Thus, *Physarum* has been used as a model for studying many of the basic processes present in many organisms, including cell differentiation, cell cycle regulation, mitosis, meiosis, cytoskeletal rearrangement, and cytoplasmic streaming.

In addition, the ability of *Physarum* to navigate using relatively complex strategies to find food and to form networks between different sources of food has drawn the attention of people outside traditional areas of biological research, including those who study networks and artificial intelligence.

Physarum polycephalum life cycle

During its life cycle, *Physarum polycephalum* transitions into different forms, some of them haploid, others diploid, and some designed to protect the organism under harsh conditions. The organism's transition from one form to another is most often triggered by the conditions of its environment, including the presence or absence of other *Physarum*.



Physarum polycephalum life cycle

Plasmodial form

In the classroom, *Physarum* is most often observed in its diploid, plasmodial form. In its plasmodial form *Physarum polycephalum* exists as a single large cell containing multiple diploid nuclei that replicate their DNA and divide synchronously. These single cells are capable of becoming very large.

Sporulation

When a plasmodium is starved and then exposed to light, it will sporulate. In nature, the organism has been observed to sporulate after climbing out of leaf litter to where it gets exposed to light. These small, dark spores can survive for many years. Thus, the formation of spores is one way the organism ensures that it will survive harsh conditions until more favorable living conditions return. Sporulation is also the first step in sexual reproduction.

During early sporulation the organism forms fruiting bodies, which initially appear as bumps emerging from the surface of the plasmodium. These bumps develop into stalk-shaped structures that project from the plasmodium surface. When viewed under the microscope they are quite dramatic. A fully developed spore contains only a single haploid nucleus.

Physarum polycephalum sporulating

Amoebas and flagellates

When there is sufficient moisture, haploid, single nucleus amoebas emerge from the spores. In contrast to what occurs in the plasmodial form, mitosis in the amoeboid form is accompanied by cell division. Repeated division of the *Physarum* amoeba results in a colony of amoebas that are genetically the same.

The amoeba can change into 2 different forms, flagellates (cells with 2 flagella) or cysts. The transformations from amoeba to flagellate and from amoeba to cyst are both reversible. If an amoeba runs out of food or encounters other adverse conditions—for example, dryness—it forms a cyst with protective walls. When the conditions become more favorable again, an amoeboid cell reemerges from the cyst. Transformation to a flagellate occurs under wet conditions. Flagellates will transform back into amoebas under drier conditions. The amoeba's ability to make these transformations allows the organism to survive a broader range of conditions than it would otherwise.

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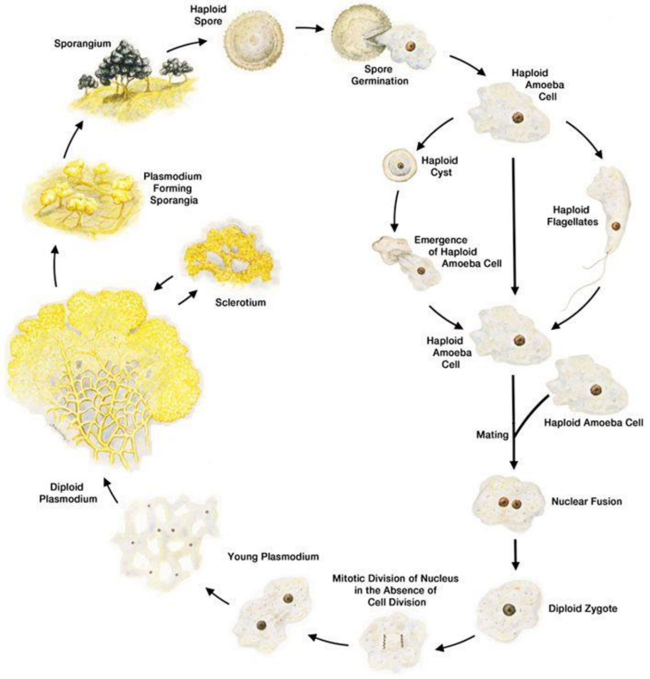


Physarum polycephalum life cycle

Sexual reproduction in slime mold

Genetic diversity is beneficial to the long-term survival of a species. As is true for most organisms, sexual reproduction is one of the mechanisms generating genetic diversity in *Physarum polycephalum*. The combining of genetic material from 2 different organisms occurs when 2 haploid amoebas fuse with each other to form a single organism with a single diploid nucleus. This single diploid cell then goes on to develop into a plasmodium. The mating of the 2 amoebas is controlled by several multi-allele mating loci.

Sclerotium

Physarum polycephalum when in the amoeboid form can respond to stressful conditions by forming a cyst. When in the plasmodial form, *Physarum* responds to starvation and light by sporulating. However, if the stressful conditions occur in the absence of light, the plasmodium will form a sclerotium, a collection of macrocysts (also called spherules) surrounded by a hard, dry protective layer. Sclerotia can form in response to a number of stimuli, including starvation, dryness, cold, low pH, and solutions with high osmotic pressure and exposure to some heavy metals. Depending upon the conditions, a sclerotium can be revived to the active form of the organism for months after it forms.

Foraging and streaming

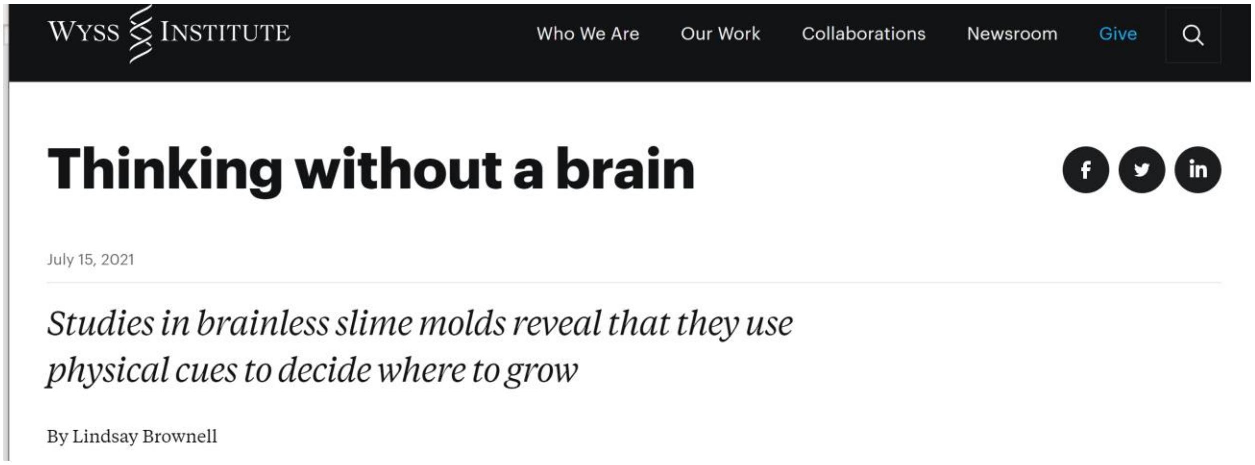
Physarum in the plasmodial form uses phagocytosis to ingest its food, which consists of small particles of organic material, bacteria, and other microorganisms. It also secretes enzymes to break down materials, which are then absorbed by pinocytosis. As it forages, the plasmodial form of *Physarum* moves around using a slow flowing movement. The part of the organism that is at the forefront of foraging has a fanlike configuration. As the organism searches for and takes in food, the cell contents stream back and forth at approximately 60-second intervals through a network of vein-like looking tubes. A single vein can be up to 1 mm in diameter, and streaming can be easily observed using a stereomicroscope. Absorbed material gets distributed throughout the cell using this mechanism. The network of tubes is reorganized as the organism moves in search of food.

The periodic streaming is accomplished through the creation of hydraulic pressure gradients. Contraction of the actomyosin network within the plasmodium creates these pressure gradients. The actomyosin network is part of an extensive network of microfilaments that exists throughout the entire plasmodium. Multiple other proteins are associated with this network. As a group, these proteins are referred to as actin-binding proteins and play a role in carrying out the functions of the actomyosin network, including its reorganization.

Foraging strategies of *Physarum*

The strategies used by the plasmodial form of *Physarum* to find food are surprisingly complex. At a basic level, the organism senses food at a distance, detects when it moves closer to or farther away from the food, and adjusts its movement according to the input it receives. This is a simple example of chemotaxis. However, the organism has at least one additional strategy for optimizing how it searches an environment for food. When seeking food, the plasmodium avoids any areas it has explored until it has covered all unexplored areas. This behavior is thought to increase the efficiency of its foraging. This type of foraging strategy has long been observed and studied in more complex organisms with internal neurologic memory that can be used to remember where the organism has been. However, *Physarum polycephalum* is a unicellular organism without a neural network for remembering. In the absence of a neurological memory, the organism creates a type of external memory by leaving a slime trail in areas from which it withdraws. The organism then strongly avoids areas with a slime track until it has explored all areas without residual slime or unless a new food source is placed in the slime-covered area.

In addition, as part of its foraging behavior *Physarum* can form networks connecting multiple food sources. These networks are similar to those created by humans with respect to efficiency. For example, a research group interested in the cost, efficiency, and resilience of networks hypothesized that a biological organism such as *Physarum polycephalum,* which has had its network forming strategies honed by years of natural selection, may provide good inspiration for the creation of networks that optimally balance these 3 characteristics. The research group created a model of the Tokyo area for *Physarum polycephalum* by placing food sources at the places representing the locations of major cities along the Tokyo rail network. Geographical features that had constrained the building of rail lines were represented as illuminated areas that would similarly be avoided by network-forming *Physarum*. The network built by the *Physarum* placed in this representative model was very similar to the existing rail line network. In addition,
the organism can make relatively complex decisions regarding its diet. Plasmodia with access to patches of food, which varied with respect to the carbohydrate-to-protein ratio and in the concentrations of each, migrated to the patch that provided the optimal diet.



Thinking without a brain

July 15, 2021

Studies in brainless slime molds reveal that they use physical cues to decide where to grow

By Lindsay Brownell

(BOSTON) — If you didn’t have a brain, could you still figure out where you were and navigate your surroundings? Thanks to new research on slime molds, the answer may be “yes.” Scientists from the Wyss Institute at Harvard University and the Allen Discovery Center at Tufts University have discovered that a brainless slime mold called *Physarum polycephalum* uses its body to sense mechanical cues in its surrounding environment, and performs computations similar to what we call “thinking” to decide in which direction to grow based on that information. Unlike previous studies with Physarum, these results were obtained without giving the organism any food or chemical signals to influence its behavior. The study is published in Advanced Materials.

“People are becoming more interested in Physarum because it doesn’t have a brain but it can still perform a lot of the behaviors that we associate with thinking, like solving mazes, learning new things, and predicting events,” said first author Nirosha Murugan, a former member of the Allen Discovery Center who is now an Assistant Professor at Algoma University in Ontario, Canada. “Figuring out how proto-intelligent life manages to do this type of computation gives us more insight into the underpinnings of animal cognition and behavior, including our own.”

Slimy action at a distance

Slime molds are amoeba-like organisms that can grow to be up to several feet long, and help break down decomposing matter in the environment like rotting logs, mulch, and dead leaves. A single Physarum creature consists of a membrane containing many cellular nuclei floating within a shared cytoplasm, creating a structure called a syncytium. Physarum moves by shuttling its watery cytoplasm back and forth throughout the entire length of its body in regular waves, a unique process known as shuttle streaming.

“With most animals, we can’t see what’s changing inside the brain as the animal makes decisions. Physarum offers a really exciting scientific opportunity because we can observe its decisions about where to move in real-time by watching how its shuttle streaming behavior changes,” said Murugan. While previous studies have shown that Physarum moves in response to chemicals and light, Murugan and her team wanted to know if it could make decisions about where to move based on physical cues in its environment alone.

It’s all relative

The researchers experimented with several variables to see how they impacted Physarum’s growth decisions, and noticed something unusual: when they stacked the same three discs on top of each other, the organism seemed to lose its ability to distinguish between the three discs and the single disc. It grew toward both sides of the dish at roughly equal rates, despite the fact that the three stacked discs still had greater mass. Clearly, Physarum was using another factor beyond mass to decide where to grow.

To figure out the missing piece of the puzzle, the scientists used computer modeling to create a simulation of their experiment to explore how changing the mass of the discs would impact the amount of stress (force) and strain (deformation) applied to the semi-flexible gel and the attached growing Physarum. As they expected, larger masses increased the amount of strain, but the simulation revealed that the strain patterns the masses produced changed, depending on the arrangement of the discs.

"Imagine that you are driving on the highway at night and looking for a town to stop at. You see two different arrangements of light on the horizon: a single bright point, and a cluster of less-bright points. While the single point is brighter, the cluster of points lights up a wider area that is more likely to indicate a town, and so you head there," said co-author Richard Novak, Ph.D., a Lead Staff Engineer at the Wyss Institute. "The patterns of light in this example are analogous to the patterns of mechanical strain produced by different arrangements of mass in our model. Our experiments confirmed that Physarum can physically sense them and make decisions based on patterns rather than simply on signal intensity."

The team's research demonstrated that this brainless creature was not simply growing toward the heaviest thing it could sense – it was making a calculated decision about where to grow based on the relative patterns of strain it detected in its environment.

"Our discovery of this slime mold's use of biomechanics to probe and react to its surrounding environment underscores how early this ability evolved in living organisms, and how closely related intelligence, behavior, and morphogenesis are. In this organism, which grows out to interact with the world, its shape change is its behavior. Other research has shown that similar strategies are used by cells in more complex animals, including neurons, stem cells, and cancer cells. This work in Physarum offers a new model in which to explore the ways in which evolution uses physics to implement primitive cognition that drives form and function," said corresponding author Mike Levin, Ph.D., a Wyss Associate Faculty member who is also the Vannevar Bush Chair and serves and Director of the Allen Discovery Center at Tufts University.

The research team is continuing its work on Physarum, including investigating at what point in time it makes the decision to switch its growth pattern from generalized sampling of its environment to directed growth toward a target. They are also exploring how other physical factors like acceleration and nutrient transport could affect Physarum's growth and behavior.

Figuring out how proto-intelligent life manages to do this type of computation gives us more insight into the underpinnings of animal cognition and behavior, including our own.

"This study confirms once again that mechanical forces play as important a role in the control of cell behavior and development as chemicals and genes, and the process of mechanosensation uncovered in this simple brainless organism is amazingly similar to what is seen in all species, including humans," said Ingber. "Thus, a deeper understanding how organisms use biomechanical information to make decisions will help us to better understand our our own bodies and brains, and perhaps even provide insight into new bioinspired forms of computation." Ingber is also the Judah Folkman Professor of Vascular Biology at Harvard Medical School and Boston Children's Hospital, and Professor of Bioengineering at the Harvard John A. Paulson School of Engineering and Applied Sciences.

Additional authors of the paper include Daniel Kaltman, Paul Jin, Melanie Chien, and Cuong Nguyen from the Allen Center for Discovery at Tufts University, Ramses Flores from the Wyss Institute, and Anna Kane, Ph.D., from both the Allen Center and the Wyss Institute.

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REVIEW

Thoughts from the forest floor: a review of cognition in the slime mould *Physarum polycephalum*

Chris R. Reid[1]

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Sensing

As a free-living organism, the plasmodium is equipped with a range of surface receptors and internal mechanisms for sensing and responding to the world around it. One of the key response mechanisms, underpinning the majority of cognitive, locomotive and homeostatic functions, is oscillation (Durham and Ridgway 1976). The plasmodium can be considered a connected mass of multitudes of tiny oscillating units, capable of expanding and contracting in response to local sensory stimuli via actin-myosin interactions—the same contractile mechanism in human muscle tissue. When cell surface receptors detect attractants such as food and moisture, oscillation frequency increases in the local area, which decreases cell surface tension, making the plasmodium more fluid (Ueda et al. 1980). This causes protoplasm to flow towards the stimulus area, and directs the movement of the entire cell. Repellents such as light and certain salts induce the opposite response, increasing the local stiffness of the cell and restricting further flow into the area.

Much of the cognitive behaviour observed in Physarum is fundamentally a consequence of communication between the myriad contractile units. While each unit senses and responds to the environment around it, physical coupling between adjacent oscillators entrains them to each other's frequencies (Nakagaki et al. 1999). This means they can respond to and influence the behaviour of their neighbours, and transfer information about the quality of local environments to distant parts of the cell. For instance, in the construction of efficient tubule networks between multiple food sources (discussed in further detail below), attractants such as food and moisture are locally sensed, resulting in increased oscillation frequency in nearby contractile units. Physical coupling results in waves of contraction that propagate outwards from the stimulus area and communicate information between proximal and distal parts of the organism. Network tubules that lie perpendicular to the direction of contractile propagation receive a lower flux of protoplasm and thus begin to decay, while parallel tubes receive more protoplasm and are reinforced and thickened. The tubules able to accommodate the highest flux are those that link the network via the shortest path; network length is thereby optimised via a positive feedback loop (Nakagaki et al. 2000; Tero et al. 2006). This simple method of local communication illustrates a form of distributed collective behaviour that leads to the emergence of sophisticated properties at the organism level (Reid and Latty 2016).

More recent work has focussed on the evidence for a biochemical signal propagated through the organism by the peristaltic waves themselves (Alim et al. 2017). A signalling molecule (later called a 'softening agent' (Kramar and Alim 2021)) was found to travel along with the internal flow, increasing contraction amplitude as it travels, and thereby facilitating its own transport via positive feedback. The signalling molecule is unknown but is likely to be either ATP or calcium ions, which are known to be vital for actomyosin interactions. It is important to note that a self-reinforcing signalling molecule could only work for attractant responses in Physarum, which induce fluidity, and not for responses to repellents, which increase stiffness. There is no reason that biochemical and coupled-oscillator models should be mutually exclusive, however, and most likely the two mechanisms are both exploited in Physarum's toolkit for sensing and responding to the world.

Due to Physarum's early popularity as a model for cell motility, there is a host of previous studies into positive and negative taxes in plasmodia responding to gradients of carbohydrates, proteins, amino acids, free nucleotides, volatile organic chemicals, salts, pH, light, humidity and temperature (Fig. 1c; (de Lacy Costello and Adamatzky 2014; Chet et al. 1977; Kincaid and Mansour 1978; Knowles and Carlile 1978; Ueda et al. 1980). There is also evidence that Physarum can sense and respond to the direction of gravity (geotaxis (Wolke et al. 1987)), magnetic fields (magnetotaxis (Shirakawa et al. 2012)) and even use mechanosensation to detect heavy masses at long-range (Murugan et al. 2021). Thus, a broad base of literature supports Physarum's ability to sense, and adaptively respond to, diverse information acquired from its environment.

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Orientation and navigation

While taxis responses (such as chemotaxis) and navigation both involve an organism moving from one place to another, the taxis examples above require only the ability to orient in space. For a macroscopic organism such as Physarum, the ability to sense an environmental gradient and move along it is a relatively simple feat. Many organisms are faced with the cognitive challenge of moving along a route between multiple points, and Physarum can achieve this in two ways. The first is conventional navigation, where an entire plasmodium must migrate through space to reach a distant point. I would argue, however, that the slime mould's method of exploratory network construction, followed by optimisation of the path between points of interest, is analogous to path-planning strategies utilised by autonomous systems, and hence can be classified as navigation.

Mazes have a long history of use in animal cognition experiments, and these are framed almost exclusively in a learning context (reviewed in Kabadayi et al. 2018). Physarum too has been tested in classic labyrinth mazes requiring the whole organism to move or extend through the maze towards a goal. However, these have never been framed in terms of learning (individuals are never challenged with solving the same maze more than once) and are always investigations of Physarum's navigational ability. For instance, Adamatzky (2012) placed a food source at the centre of a circular labyrinth maze with an agar floor, challenging the plasmodium to navigate towards the goal. In this case, the walls of the maze extended below the level of the agar, creating a distinct channel of agar along which chemoattractants can diffuse from the centre to the plasmodium inoculation site. It is therefore no surprise that the slime mould simply follows this gradient, and in doing so follows the shortest path through the maze.

The more commonly researched example of navigation is experimentally framed around Physarum's excellent ability to construct efficient networks between multiple points of interest. The plasmodium spreads itself out in search of food (exploration), often finding multiple food sources simultaneously some distance apart. This stage is analogous to the slime mould building a map of its surroundings, with the plasmodial network itself forming the many potential routes between food sources A and B. Having found multiple food sources, the plasmodium now seeks to engulf them with biomass, while also staying connected as a single entity (exploitation). This fundamental trade-off has resulted in strong selection for shortest-path-finding strategies in Physarum, which has been a major focus of slime mould behavioural research within the last two decades.

The landmark study which single-handedly spurred this flurry of research activity is a brief communication in *Nature* by Nakagaki and colleagues (2000), in which Physarum plasmodia were spread through a labyrinth maze connecting two food sources, forming a single cell in the shape of the maze (Fig. 1d). Over time, the plasmodia retracted biomass from the dead ends and longer paths through the maze, until eventually a single tubule remained, tracing out the single solution. As stated by the authors, "This remarkable process of cellular computation implies that cellular materials can show a primitive intelligence." (Nakagaki et al. 2000). These humble words sparked a revolution, and a host of studies followed, exploring the capabilities of Physarum in network optimisation (Nakagaki et al. 2001, 2004; Reid and Beekman 2013), and building mathematical models based on empirical insights (Tero et al. 2006, 2007).

These studies have been of particular interest to network engineers seeking new methods for designing optimisation algorithms. Human-designed networks, such as those for telecommunication or transport, tend to place a high priority on shortest paths, as these provide the quickest travel time through the network, and the lowest construction cost. These networks are, however, the most at risk of catastrophic failure after even the slightest disruption, so natural selection has favoured biological networks that find a trade-off between path efficiency and the robustness of additional redundant links (Middleton and Latty 2016). A landmark study produced by Nakagaki's lab (Tero et al. 2010) sought to extract network design rules from Physarum that specifically encoded an optimal trade-off between shortest-path efficiency and robustness. The researchers allowed Physarum to explore an agar plate in the shape of the Tokyo district, with food sources placed in the locations of railway stations (Fig. 1e). As the slime mould pruned its network to connect these 'stations' (over the course of approximately 26 h), the resulting networks had comparable efficiency, fault tolerance and cost to the existing Tokyo Railway infrastructure. Moreover, the authors were able to use the behavioural observations of Physarum network construction to build mathematical models for network growth, with tuneable parameters for adjusting features such as fault tolerance and transport efficiency. These could be useful for engineers designing future transport networks, or for guiding the development of self-organised networks such as remote sensor arrays, wireless mesh networks, or the Internet-of-Things (Tero et al. 2010; see also Gao et al. 2019 for an extensive review of Physarum-inspired models and computations, and their impacts).

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Decision-making

Decision-making is defined here following Reid et al. (2015) as "the action by an entity of selecting an option from a set of alternatives, based on characteristics of the alternatives that the entity can perceive." The utility of this definition is that it relies only on the observable actions of the organism in question, and makes no assumption of underlying mechanisms. As Physarum can clearly perceive a vast array of information about its environment, it is no surprise that plasmodia consistently choose the better of two presented options when they differ in only a single attribute, such as caloric concentration (Latty and Beekman 2010), temperature (Durham and Ridgway 1976) or light levels (Latty and Beekman 2011b). Indeed, most of these decisions can be made using simple taxis responses. An exception is a study of length discrimination (Mori and Koaze 2013). When presented with the option of connecting two food sources via either a short or long route in a circular arena, Physarum predictably chose the shorter route. This choice was consistent despite changing the diameter of the arena, indicating that Physarum bases its decision on the ratio of the two lengths, rather than any absolute difference. This pattern of cognition of difference in stimulus magnitude (constrained by Weber's law (Fechner 1948)) is consistently observed in decision-making systems from humans (Deco et al. 2007) to other mammals (Yoshioka 1929), birds (Dixit et al. 2022) and insects (Perna et al. 2012).

Similarly, when examining the decision to remain within a food patch or explore elsewhere, both Physarum and another plasmodial slime mould (*Didymium bahiense*) were found to use incremental patch-departure heuristics, just as insects and humans do (Latty and Beekman 2015). Plasmodia were inoculated inside a grid of circular food discs of either high or low quality, and the time taken to leave the patch was recorded as a function of the number and quality of food sites sampled. Importantly, chemosensory cues of patch quality were precluded by placing each of the food sources on non-permeable plastic circles. Furthermore, the experimental plates were designated as either 'safe' (darkened) or 'risky' (well lit) environments. Physarum tended to stay longer within a patch if they had recently experienced high-quality food, and within darkened, 'safe' patches. *D. bahiense* tended to remain within a patch if it had recently encountered food of any quality and did not alter its strategy in dark or lit environments. Studies such as this highlight the utility of cognitive paradigms applied across (and within) broad taxa, as well as outlining a rich future avenue of research into why and how these diverse strategies exist, even between two species of plasmodial slime mould.

In the interests of understanding cognition, it is necessary to explore more difficult decision-making scenarios, such as when multiple attributes per option can be evaluated independently, and when several of these attributes may conflict with each other. These so-called 'multi-attribute compensatory problems' are the gold standard, because they require the organism to compare options based on their relative differences, integrating information along multiple axes of 'quality', rather than simply whether one attribute exceeds a desired threshold (see Reid et al. (2015) for a detailed discussion of decision-making in Physarum and other non-neural organisms).

Physarum is capable of making trade-offs between exploitation of food and exposure to danger. For instance, when choosing between high-quality food (positive stimulus) that is illuminated with strong light (negative stimulus), and an alternative option of low-quality food in the dark, Physarum will choose the safer, low-reward option. However, if the risky option is at least five-fold higher in food concentration than the safe option, Physarum will gamble on the illuminated food source (Latty and Beekman 2010). When forced to build a network connecting two food sources that passes through an intensely lit region, Physarum will make the optimum trade-off of path efficiency and exposure to light (Nakagaki et al. 2007). The single tubule connecting the food sources travels along a deflected path that occupies more space within the dark region when the lit region has a higher photo-intensity—a geometric feature similar to the path of light travelling through two materials with different refractive indices (Nakagaki et al. 2007). Importantly, the tubule path did not differ between totally dark and totally lit controls, indicating the amount of deflection is determined entirely by Physarum's computation of the ratio of light intensity between the two regions.

While most animal cognition studies focus on adaptive decision-making, where the outcome of the decision-making process is a beneficial response that increases the organism's fitness, it can be useful to examine maladaptive cases, such as irrational decision-making. Previous explanations of irrationality have centred around neurological mechanistic explanations, such as dopamine-reward systems (Anselme and Güntürkün 2019; Cocker et al. 2012), or specific brain regions such as the ventromedial prefrontal cortex (Koenigs et al. 2007). However, evidence from Physarum suggests that even non-neural organisms can be irrational. When Physarum plasmodia choose between two options that vary equally in two competing attributes—food concentration and light intensity—they show no preference for one option over the other. However, when a third, inferior option is introduced, plasmodia change their preference (Latty and Beekman 2011a). This is irrational because the decoy option should not affect the organism's choice. In a related study, the same researchers showed that Physarum plasmodia are subject to speed/accuracy trade-offs when faced with difficult decisions (Latty and Beekman 2011b), another phenomenon that has been traditionally explained using a neuroscientific lens (Bogacz et al. 2010; Chittka et al. 2009).