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Habituation and other complex processes in *Physarum Polycephalum*: what a single-celled organism can teach us about how we learn

BACKGROUND:

In the soil of any given temperate forest across the globe there grows a group of ancient, otherworldly, and fascinating amoebas that are capable of remarkably sophisticated behaviors. While naturalists have known of these creatures' existence for centuries, scientists are just now starting to explore their versatility and intelligence. With huge variations between species, no brain or neurons to speak of, and creative nicknames such as 'dog's vomit,' these organisms make up one of the most unique biological communities in the world; the slime molds.

'Slime mold' is a rather informal umbrella term used to describe over 900 different species worldwide of eukaryotic organisms that are not ancestrally related. This is called a polyphyletic grouping, meaning that they are placed together based on common characteristics instead of evolutionary derivation. Half accurate descriptor and half misnomer, the term 'slime mold' was coined due to the gelatinous texture these organisms take during certain stages in their life cycles and because they were originally incorrectly classified under the subkingdom Gymnomycota as Fungi. It's no surprise that slime molds were classified as fungi: they are often spotted in forests pulsing along on plants and decaying wood, shooting up spores that look very similar to the fruit of a fungus. On any given piece of decaying organic matter there lies a cornucopia of microbes, which is a slime mold's ideal feeding ground. No matter how alike they look at first glance, slime molds and fungi are completely separate organisms with very different natural processes. A century ago, Fungi were classified as organisms that weren't as small as bacteria, did not have green pigment chlorophyll, and could not move (Utah State University). Today, organisms in the kingdom Fungi are defined by the same previously mentioned principles with the additional rule that their cell walls contain chitin. Slime molds move, lack chitin in their cell walls, and perform phagocytosis regularly. Fungi produce enzymes that break down organic matter so that it can be absorbed through their cell walls, showing yet another reason slime molds don't belong in the same clave as any fungus. Slime molds are now rightfully classified within the ambiguous kingdom Protista and under the phylum Amoebozoa.

Within Amoebozoa, slime molds compose the polyphyletic group Mycetozoa. There are three major types of slime molds within Mycetozoa: Protostelids, Dictyostelids, and Myxogastria. Protostelids are the least studied of the three, and are microscopic amoeboid organisms that form simple fruiting bodies consisting of a cellular stalk and a couple spores. Unlike dictyostelids and myxogastria, protostelids have not yet been used as a model organism to study a specific system (Ha et al., 2021).

Dictyostelids are cellular slime molds, which spend most of their lives as individual unicellular protists but can follow each other's chemical signals to coagulate and form one multicellular cluster that acts as a single organism. Dictyostelids are extremely useful in the study of cell movement and mobility, and many researchers love to examine them. Some scientists fondly refer to the organisms as "Dicty," and even send their fastest cultures to Harvard to race each other for a small prize and bragging rights (Hotz, 2014). *Dictyostelium discoideum* has been previously studied to understand cell differentiation, because it has both single- and multi-celled life stages with the cells showing some degree of differentiation in the multicelled form (Ha et al., 2021). *Dictyostelium* can identify when sufficient cell density has been reached to warrant transition from growth to differentiation, as well as when exogenous nutrients become limiting. Using pulses of an aggregation signal (cAMP), *Dictyostelium* can synchronously begin development (Loomis, 2014). *Dictyostelium* can also recognize kinship, and will only continue development when surrounded by relatives. John Bonner, an American biologist and lover of cellular slime molds, procured the first solid evidence for chemotaxis in eukaryotes in a 1947 experiment using *Dictyostelium*. Bonner showed that starving *Dictyostelium* cells secreted a chemoattractant. Other *Dictyostelium* cells in a downstream environment responded to this attractant by traveling up a gradient (Bonner, 1947).

The pseudo-plasmodial structures formed by dictyostelids, called "slugs," were once thought to be lead in assemblage by a specific leader-cell, but recent research has proven that they have no distinct order. This is impressive when considering how effectively these single-cells can aggregate. Cellular slime mold aggregation has been observed to create aggregation algorithms which have been used in computer modeling and even in video games to control the behavior of non-player characters. Evelyn Fox Keller and Mitch Resnik, two scientists at the Massachusetts Institute of Technology, developed the first set of equations from cellular slime molds and created StarLogo, an agent-based simulation programming language. StarLogo is an environment used by students and teachers to make 3D games and simulations for understanding complex systems, all modeled on the movements of cellular slime molds.

Myxogastria, also referred to as myxomycetes, are the third subgroup within Mycetozoa and are considered the "true" or plasmodial slime mold. Plasmodial slime molds are single cells, essentially bags of cytoplasm with thousands of individual nuclei inside and are visible to the naked eye. These "supercells," or syncytium, are heterokaryotic meaning their nuclei are genetically different from each other. Slime mold nuclei divide in synchronism with each other, multiplying at the same time. This synchronous division is advantageous in conservation of energy and in maintaining the same ratio of genetic diversity between nuclei. Of all Mycetozoa, Myxomycetes are the most commonly observed, and of all Myxomycetes, the cellular slime mold *Physarum polycephalum* is by far the most researched.

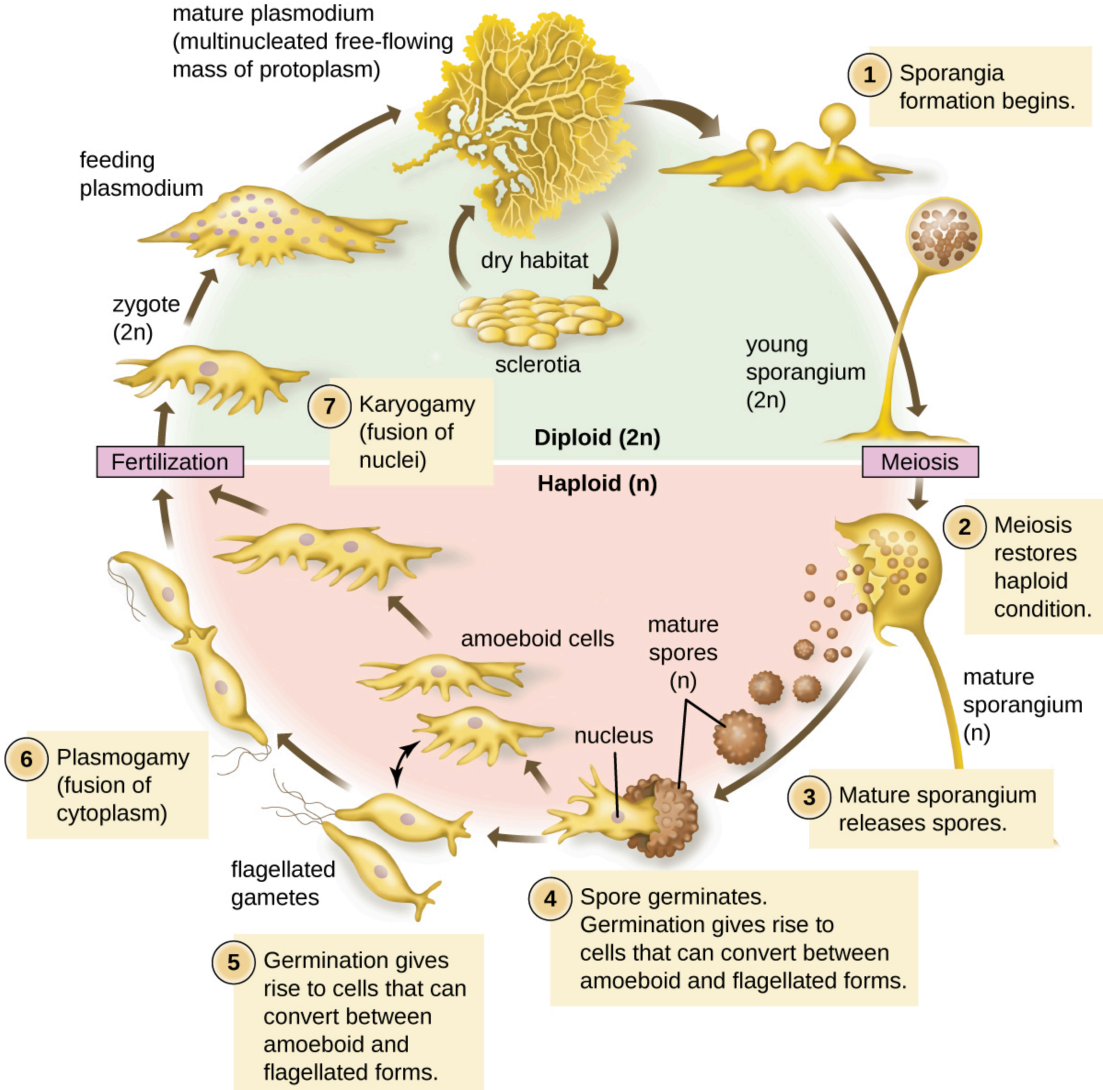


Fig. 1 Life cycle of *Physarum polycephalum* (Ha et al., 2021)

PHYSARUM POLYCEPHALUM:

Physarum polycephalum means "many-headed slime mold," which fits this organism's appearance as it searches for food by spreading probing, pseudopodial arms out across its landscape. A pseudopod, or pseudopodium, is a temporary arm-like appendage of a eukaryotic cell membrane that develops in the direction of movement. In this amoeboid fashion, *Physarum* crawls its way through the natural detritus and decaying matter on its forest floor environment. Perhaps it's better to describe *Physarum's* movement as a pulse rather than a crawl - actin and myosin, the same muscle proteins that humans have, behave as a kind of network to contract and relax cytoplasm. This strategic protoplasmic streaming successfully moves the amoeba at a speed of about 4 cm/hour and helps to distribute nutrients throughout the body (Matsumoto et al., 2008). Periodic streaming is accomplished through the creation of hydraulic pressure gradients. Actin and myosin create the actomyosin, an extensive network of filaments throughout the entire plasmodium. Contraction of the actomyosin is what creates the different pressure gradients necessary for streaming and movement.

Physarum polycephalum has a diplontic lifestyle, meaning that its main life stage is diploid and it cycles through a haploid stage. Transitions between these two forms are typically triggered by external or environmental factors, including the presence of other *Physarum*. When a plasmodium is starved and exposed to light, it will begin to sporulate. In its natural habitat, Physarum tends to sporulate after climbing out of the leaf litter. Spores appear as small, dark nodules on the end of stalks, and can survive for years through a myriad of unfavorable conditions. Sporulation is one way the organism ensures surviving until conditions are more favorable, and is also the first step in sexual reproduction. The early stages of sporulation involve the growth of fruiting bodies, or sporangia, which initially appear as bumps emerging from the plasmodium. (Fig 1, stage 1) Inside the sporocarp, the nuclei undergo meiosis to produce haploid spores. A fully developed spore only has a single haploid nucleus, thus pushing the plasmodium into the haploid stage of its life (Fig 1, stage 2). With sufficient moisture in the environment, the spores will germinate, and from their shells new amoebas will grow (Fig 1, stage 4). Mitosis, in this early amoeboid stage, is accomplished by cell division. These infant amoebas have a single nucleus, and can convert between amoeboid and flagellated gamete forms. This transformation is a reversible adaptation designed to help the organism survive different environmental conditions. In a wet environment, the amoeba will transform into a flagellate. When conditions become dryer, the flagellate will transform back into an amoeba. Most species on earth have two different sized gametes, one being large and immoble, the other being small and mobile. Slime mold's sex cells are isogamous, meaning they have only one kind of gamete.

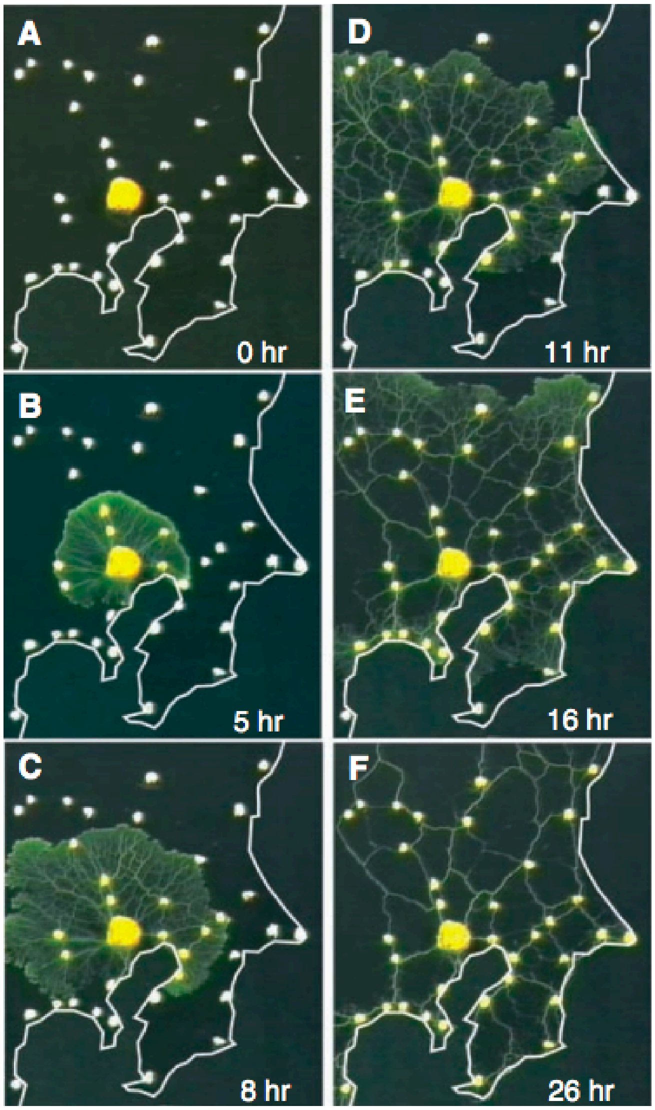
In order to maintain high levels of genetic diversity, *Physarum,* like most other organisms, must sexually reproduce. In the case of this Myxomycete, sexual reproduction occurs when two different haploid amoebas fuse with each other through cell fusion to create a single organism with a diploid nucleus, which goes on to develop into a plasmodial form. Slime mold sexes are a bit more complicated than that of human sexes. The sex cells within *Physarum* carry three variable genes, *matA, matB,* and *mat,* all of which contain variants. Each sexually mature plasmodium carries two copies of each of these genes and is capable of producing 8 types of sex cells. When taking into account all the variants for the variable genes, there's the potential for over 500 different sexes (*Sexes of Slime Molds* 2019). Successful reproduction occurs when each partner finds a cell with a different variation of the three genes, and with so many possible combinations, it's nearly always possible.

COMPUTATIONAL USES:

Even though *Physarum polycephalum* is a unicellular organism, many of its lifecycle processes are similar to those of complex organisms. Because of this, *Physarum* is a very popular subject used to study cell differentiation, cell cycle regulation, mitosis, meiosis, cytoskeletal rearrangement, and cytoplasmic streaming. *Physarum's* foraging patterns and behaviors are surprisingly complex. At the most basic level, the organism senses food at a distance and adjusts course depending on whether or not its pseudopods reach closer to or further away from the food source. Neural organisms capable of memory have an easy time remembering not to go back to where they have already explored, but *Physarum* can do the same by leaving a slime trail over places it has previously foraged. This behavior increases the efficiency of *Physarum's* foraging, and could even be considered an external form of memory. *Physarum* is well known outside of biological circles, as the plasmodium's foraging patterns have an unexpected computational ability. *Physarum*'s network of pseudopods has also evolved to find the most efficient pathway between food sources, which means that when data is represented by attractants and repellents, the plasmodial foraging choices can be seen as computations.

There have been many fascinating computational studies done on *Physarum*, most famously the 2010 transportation network study in which physarum polycephalum demonstrated comparable efficiency to human-made infrastructure networks by recreating the Tokyo railway system (Nakagaki et al., 2010). (Fig 2 & 3) Similar studies on geographical problems and evaluations of infrastructure and transportation systems have been done using *Physarum* in other countries as well. In the United Kingdom it's been used to find the optimal layout of transportation links in urban areas (Adamatzky & Jones, 2010). *Physarum* has been used to approximate Mexican highways (Adamatzky et al., 2010), as well as Australian (Adamatzky & Prokopenko, 2011), Iberian (Adamatzky & Alonso-Sanz, 2011), and Dutch motorways (Adamatzky et al., 2013).

Similar to its cousin *Dictyostelium, Physarum's* foraging behavior has been implemented in emerging video game rendering technology. The pseudopod formations have been used to compute planar polygons, which are a classical problem of modern computational geometry (Adamatzky, 2011). These planar point calculations are used in the simulated graphics of movies and video games, and the faster the calculations can be done the smoother and more realistic the graphics become. *Physarum* has also been used to optimize an algorithm to solve minimal exposure path problems corresponding to worst-case coverage in wireless sensor networks (Liu et al., 2012). It's been used to solve computational problems with natural parallelism (Adamatzky, 2007) and natural spatial logic (Schumann & Adamatzky, 2011), and to solve logical-computing schemes (Tsuda et al., 2003).



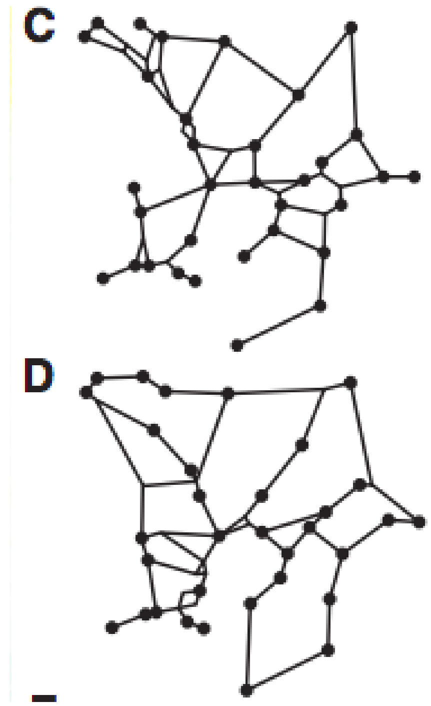


Fig. 2, left, growth of *Physarum polycephalum* over the span of 26 hours. Food sources are set up to emulate high population areas in Tokyo. **Fig. 3,** right, resulting *Physarum* network **(C)** compared with Tokyo's existing network **(D)**. Both images taken from Nakagaki et al. "Rules for Biologically Inspired Adaptive Network Design" (Nakagaki et al., 2010)

Physarum's pseudopodial tube morphogenesis has been studied to find shortest path computations (Nakagaki et al., 2001). Toshiyuki Nakagaki, a slime mold expert at Hokkaido University who was integral to the previously mentioned Tokyo railway approximation, jumpstarted the recent trend in *Physarum* computational research in 2001 when he demonstrated its ability to solve a maze (Nakagaki, 2001). Physarum has even been used to approximate the development of ancient Roman road networks in the Balkans (Evangelidis et al., 2017). In a more creative study, *Physarum's* electrical activity during growth was captured by electrodes and synthesized into sound (Miranda et al., 2011). The resulting noise, available on YouTube, is almost describable as avant-garde electronic jazz.

HABITUATION:

Habituation can be defined as a diminishing of a behavioral response that results from repeated stimulation unrelated to motor fatigue or sensory fatigue. Traditionally, habituation is also defined by dishabituation, or the original behavioral response returning after the stimulus has been removed (Rankin et al., 2008). Humans perform habituation all the time, for example, when we stop feeling fabric against our skin after putting on clothing, or when a specific background sound or smell becomes unnoticeable after a certain amount of time. Habituation, typically termed as the "simplest form of learning," is well-studied behaviorally but not mechanically. Surprisingly little is known about the neural mechanisms that underlie habituation behavior. The reason habituation is deemed as the simplest form of learning is because it allows for animals to filter out unimportant stimuli for just the important ones, meaning that it's the prerequisite for all other forms of learning.

In 2016, Audrey Dussutour, David Vogel and Romain P. Boisseau performed an experiment which showed *Physarum polycephalum* demonstrating habituation. This discovery made by Dussutour, Vogel, and Boisseau was monumental because it was an unequivocal report of learning being done by a single-celled organism. Previously, habituation had almost completely been studied in multicellular neural organisms.

> "We usually think of learning as a trait that is limited to organisms with brains and nervous systems. Indeed, learning is often equated with neuronal changes such as synaptic plasticity, implicitly precluding its existence in non-neural organisms. While the evolutionary benefits of learning are clear, very little is known about its origins. Even the 'simplest' organisms adapt to changing environments, raising the exciting possibility that mechanisms for learning might pre-date the evolution of nervous systems, possibly existing in a breadth of as yet unstudied organisms. Tantalizing results suggest that the hallmarks for learning can occur at the level of single cells. If true, then studies of unicellular model species displaying learning abilities promise exciting insights to the earliest manifestations of learning, a key innovation in the history of life." (Boisseau et al., 2016)

In the experiment, cultures of *Physarum* were made to cross bridges of caffeine and quinine, both stimulants for the plasmodium, in order to reach a food source. Control groups were given a bridge of plain agar. The specific mechanics of *Physarum*'s chemoreception to quinine and caffeine are unknown, but it's speculated that they bind to chemoreceptors on the cell membrane, which start a signaling cascade that results in a new migration direction (Natsume et al., 2006). For five days, slime molds crossed these bridges and on the sixth day were tested for habituation and stimulus generalization abilities. The two following days were a recovery period, and none of the slime molds were exposed to any stimulants.

Then, on the ninth day, came the final test for habituation and stimulant response. The response variables they recorded were the time for the plasmodia to cross the bridges and the size of the pseudopods. This habituation assay properly demonstrated that the slime molds stopped responding to the aversive stimulus when it was repeated, but recovered and began responding again when it was withheld during the recovery period, and that habituation was specific between the two stimuli. This response couldn't be explained by genetic inheritance as it happened within a single lifetime. Thus, *Physarum polycephalum* met the two major established criteria for habituation.

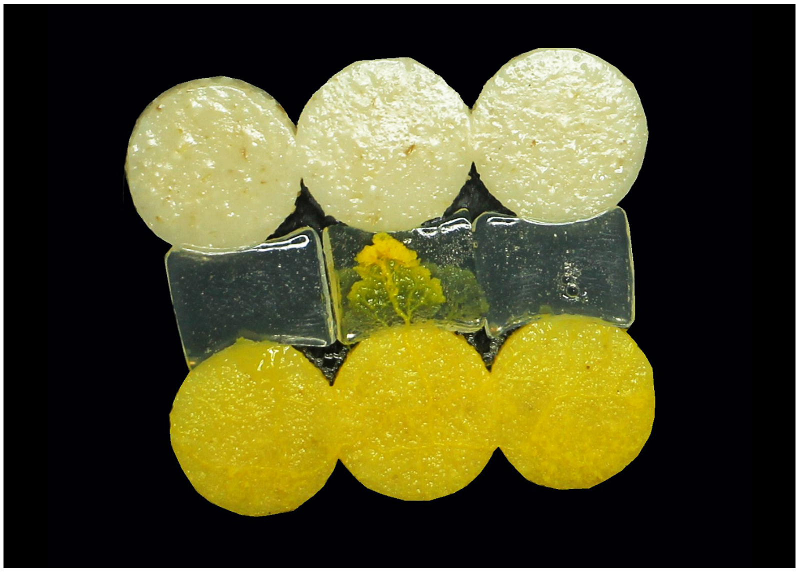


Fig. 4, *Physarum polycephalum* crossing a plain agar bridge

A follow-up study done by Vogel and Dussutour in 2016 suggests that *Physarum* can learn these habituation behaviors through some kind of communication, not just exposure. In this experiment, non-habituated, or "naive" slime molds were allowed to fuse with habituated ones, and then all were tested for habituation and stimulus resistance. Not only were the merged entities resistant to the used stimulus, but the relationship between the proportion of habituated slime molds within the merged entity and the level of habituation was non-linear. This non-linear relationship means that the observed habituation of merged entities can't just be explained by a dilution of the response due to cytoplasmic mixing. For example, a merged group of mostly unhabituated slime molds and a few habituated slime molds still showed an adaptive response. This is extremely strong experimental evidence that an adaptive response can be transferred, or communicated, through cell fusion.

Cell fusion is an important cellular process observed in all eukaryotes in which multiple cells join to form a uninucleate or multinucleate cell. During cell fusion, cells can exchange resources such as molecules or organelles. The first recorded observation of cell fusion was made by Theodor Schwann in 1847. Schwann, a German physician and physiologist, made the significant contribution to biology of proposing the cell as the basic unit of life, and that all living things are composed of cells. Despite Schwann observing cell fusion in the 1800s, it wasn't until 1960 that biologists deliberately fused cells for the first time. Cell fusion is involved in a myriad of biological processes for many animals, including mating, fertilization, immune response, and cell-mediated tissue regeneration (Chen & Olson, 2005). For most fungi and slime molds, cell fusion happens frequently, enhances foraging success (Bastiaans et al., 2015), and allows assistance from other cells through the sharing of resources (Schaap et al., 2015). Spontaneous synchronization of nucleic division after the fusion of separate *Physarum* plasmodiums suggests that there are switch-like control mechanisms spread over the entire plasmodium as a result of cytoplasmic mixing (Walter et al., 2013).

Vogel and Dutussour checked to make sure the conjoined habituated and non-habituated slime molds successfully performed protoplasmic mixing, and even tested to find the right amount of time to let them fuse. They determined that three hours was the maximum threshold because after that the merged entity would begin sending out new pseudopods. To their surprise, the first pseudopod from the joined groups to reach the food source was more often from the unhabituated side, rather than from the habituated one. In the conclusion, they made it clear that this habituating behavior is most likely a logical, evolutionary strategy to cope with environmental stress.

> "The ability of slime moulds to share an adaptive behavioural response directly via cell fusion provides a rapid and efficient means for slime moulds to adapt to their environment. Slime moulds that have been habituated to an environmental repellent can relay by fusion any potential changes in gene expression or physiology that occur during habituation. Recipient slime moulds then become pre-habituated to environmental repellents before the repellents are even encountered. Thus, fusion can confer resistance to naive slime moulds that otherwise would be susceptible to this repellent."(Dussutour & Vogel, 2016)

Dussutour and others completed yet another experiment in which they tried to narrow down the habituation mechanism by forcing habituated slime molds into their dormant sclerotia form and then reviving them again (Boussard et al., 2019). Their results showed that information acquired during the stimulus training could also be preserved in the dormant form up to a month later.

Dusuttor (pictured below holding a petri dish full of slime mold) and her colleagues have provided very strong evidence that *Physarum polycephalum* can habituate to noxious substances, but can this evidence be described as a form of primitive learning? Traditionally, scientists have always linked learning to the existence of a nervous system, and scientists aren't typically eager to break tradition. In light of *Physarum's* newfound fame, some neuroscientists in particular are objecting to what they perceive as the 'devaluing' of the human brain. Some simply take issue with the terms traditionally associated with psychology and neurobiology used in Dussutour's work, such as learning, memory, and intelligence. John Smythies, director of the Laboratory for Integrative Neuroscience at the University of California, San Diego, is particularly passionate about this semantic debate. In an Wired article he is quoted as saying "learning implies behavior and dying is not that!" Michael Levin, a biologist at Tufts University, has a much more holistic approach. In the same article, Levins said "Brains are great, but we have to remember where they came from. Neurons evolved from non-neural cells, they did not magically appear." He went on to say that "computer science long ago learned that information-processing is substrate-independent. It's not about what you're made of, it's about how you compute." (Moskvitch, 2018)

Fig. 4, Audrey Dussutour with *Physarum polycephalum*

Although the debate over *Physarum's* intelligence is much more about semantics than science, it's an interesting and important discussion to be had. No matter the semantics, slime molds should be studied further as a possible model system in which we can investigate the fundamental mechanisms of learning processes. By studying learning behaviors in single-celled organisms, we allow for a more comprehensive understanding of how the earliest manifestations of learning evolved, and a phylogenetic understanding of where in the tree of life learning first began. By picking the 'brain' of *Physarum polycephalum,* we are essentially picking our own brains. There is still much work to be done in this novel emerging field of primitive cellular cognition. To gain further recognition, researchers will need to collect more evidence with a broader range of stimuli. And, more importantly, they'll need to determine the exact mechanisms of habituation and transfer through cell fusion. The possible similarities and differences they'll find between our own neural patterns and the habits of a cellular slime mold could be astounding. The beauty will be in the discovery.

REFERENCES:

1. University US. Slime Molds. Herbarium. [accessed 2021 April 18]. https://herbarium.usu.edu/fun-with-fungi/slime-molds
2. Ha M, Morrow M, Algiers K. 5.2.1: Slime Molds. Biology LibreTexts. 2021 Apr 2 [accessed 2021 Apr 26]. https://bio.libretexts.org/Bookshelves/Botany/Botany_(Ha_Morrow_and_Algiers)/Unit_1%3A_Biodiversity_(Organismal_Groups)/05%3A_Protists/5.02%3A_Heterotrophic_Protists/5.2.01%3A_Slime_Molds
3. Hotz RL. Scientists, Start Your Dictyostelium: Researchers Rev Up for Slime-Mold Race. The Wall Street Journal. 2014 Mar 11 [accessed 2021 Apr 26]. https://www.wsj.com/articles/SB10001424052702304450904579369372368336300
4. Loomis WF. Cell signaling during development of Dictyostelium. Developmental biology. 2014 Jul 1 [accessed 2021 Apr 26]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4075484/
5. Bonner JT. Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold Dictyostelium discoideum. The Journal of experimental zoology. 1947 [accessed 2021 Apr 26]. https://pubmed.ncbi.nlm.nih.gov/20268085/
6. Paine E. Think Single-Celled Organisms Are Simple? Think Again! The Slime Mold Physarum polycephalum, a Single-Celled Organism for Student Investigations. Carolina Biological Supply. [accessed 2021 Apr 25]. https://www.carolina.com/teacher-resources/Interactive/think-single-celled-organisms-are-simple-think-again-the-slime-mold-physarum-polycephalum-a-single-celled-organism-for-student-investigations/tr41405.tr
7. Matsumoto K, Takagi S, Nakagaki T. Locomotive mechanism of Physarum plasmodia based on spatiotemporal analysis of protoplasmic streaming. Biophysical journal. 2008 Apr 1 [accessed 2021 May 4]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2267142/
8. What is actomyosin? MBInfo. 2018 Feb 6 [accessed 2021 May 4]. https://www.mechanobio.info/cytoskeleton-dynamics/what-are-contractile-fibers/what-is-actomyosin/
9. Sexes of Slime Molds. DragonflyIssuesInEvolution13 Wiki. 2019 [accessed 2021 May 4]. https://dragonflyissuesinevolution13.wikia.org/wiki/Sexes_of_Slime_Molds
10. Nakagaki T, Kobayashi R, Yumiki K, Fricker MD, Bebber DP, Ito K, Saigusa T, Takagi S, Tero A. Rules for biologically inspired adaptive network design. pubmed.gov. 2010 Jan 22 [accessed 2021 May 5]. https://pubmed.ncbi.nlm.nih.gov/20093467/
11. Adamatzky A, Jones J. ROAD PLANNING WITH SLIME MOULD: IF PHYSARUM BUILT MOTORWAYS IT WOULD ROUTE M6/M74 THROUGH NEWCASTLE. World Scientific.

2010 [accessed 2021 Apr 26].
https://www.worldscientific.com/doi/abs/10.1142/S0218127410027568

12. Adamatzky A, Martinez GJ, Chapa-Vergara SV, Asomoza-Palacio R, Stephens CR. Approximating Mexican highways with slime mould. Cornell University. 2010 Oct 4 [accessed 2021 Apr 26]. https://arxiv.org/abs/1010.0557
13. Adamatzky A, Prokopenko M. Slime mould evaluation of Australian motorways. Taylor & Francis. 2011 Aug 17 [accessed 2021 Apr 26]. https://www.tandfonline.com/doi/abs/10.1080/17445760.2011.616204
14. Adamatzky A, Alonso-Sanz R. Rebuilding Iberian motorways with slime mould. Biosystems. 2011 Apr 17 [accessed 2021 Apr 26]. https://www.sciencedirect.com/science/article/pii/S0303264711000608
15. Adamatzky A, Lees M, Sloot P. BIO-DEVELOPMENT OF MOTORWAY NETWORK IN THE NETHERLANDS: A SLIME MOULD APPROACH. Bio-development of Motorway Network in the Netherlands: A Slime Mould Approach. 2013 [accessed 2021 Apr 26]. https://www.worldscientific.com/doi/abs/10.1142/S0219525912500348
16. Adamatzky A. Slime mould computes planar shapes. Cornell University. 2011 Jun 1 [accessed 2021 Apr 26]. https://arxiv.org/abs/1106.0305
17. Liu L, Song Y, Ma H, Jang X. Physarum optimization: A biology-inspired algorithm for minimal exposure path problem in wireless sensor networks. Physarum optimization: A biologically-inspired algorithm for minimal exposure path problem in wireless sensor networks . 2012 Mar [accessed 2021 Apr 26]. https://ieeexplore.ieee.org/document/6195492
18. Schumann A, Adamatzky A. Physarum Spatial Logic. New Mathematics and Natural Computation. 2011 [accessed 2021 Apr 26]. https://www.worldscientific.com/doi/abs/10.1142/S1793005711002037
19. Adamatzky A. PHYSARUM MACHINE: IMPLEMENTATION OF A KOLMOGOROV-USPENSKY MACHINE ON A BIOLOGICAL SUBSTRATE. Physarum Machine: Implementation of a Kolmogorov-Uspensky machine on a biological substrate. 2007 [accessed 2021 Apr 26]. https://www.worldscientific.com/doi/abs/10.1142/S0129626407003150
20. Tsuda S, Aono M, Gunji Y-P. Robust and emergent Physarum logical-computing. Biosystems. 2003 Dec 30 [accessed 2021 Apr 26]. https://www.sciencedirect.com/science/article/pii/S0303264703001801
21. Nakagaki T, Yamada H, Tóth Á. Path finding by tube morphogenesis in an amoeboid organism. Biophysical Chemistry. 2001 Aug 23 [accessed 2021 Apr 26]. https://www.sciencedirect.com/science/article/abs/pii/S030146220100179X

22. Nakagaki T. Smart behavior of true slime mold in a labyrinth. Research in Microbiology. 2001 Oct 18 [accessed 2021 Mar 23]. https://www.sciencedirect.com/science/article/abs/pii/S0923250801012591
23. Evangelidis V, Jones J, Dourvas N, Tsompanas M-A, Sirakoulis GC, Adamatzky A. Physarum machines imitating a Roman road network: the 3D approach. Nature News. 2017 Aug 1 [accessed 2021 Apr 26]. https://www.nature.com/articles/s41598-017-06961-y
24. Miranda ER, Adamatzky A, Jones J. Sounds synthesis with slime mould of Physarum Polycephalum. Journal of Bionic Engineering. 2011 Jun 1 [accessed 2021 May 3]. https://link.springer.com/article/10.1016/S1672-6529(11)60016-4
25. PhysarumMachines. Physarum Music. YouTube. 2010 Oct 29 [accessed 2021 May 3]. https://www.youtube.com/watch?v=F79D_YWXycI
26. Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, et al. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiology of learning and memory. 2008 Nov 6 [accessed 2021 Apr 28]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2754195/#:~:text=Go%20to%3A-,Definition%20of%20Habituation,sensory%20fatigue%20or%20motor%20fatigue
27. Boisseau RP, Vogel D, Dussutour A. Habituation in non-neural organisms: evidence from slime moulds. Proceedings of the Royal Society B: Biological Sciences. 2016 Apr 27 [accessed 2021 Mar 13]. https://royalsocietypublishing.org/doi/full/10.1098/rspb.2016.0446#RSPB20160446C15
28. Natsume K, Miyake Y, Yano M, Shimizu H. Information Propagation by Spatio-Temporal Pattern Change of Ca2+ Concentration throughout Physarum polycephalum with Repulsive Stimulation. Cell Structure and Function. 2006 Mar 27 [accessed 2021 May 2]. https://www.jstage.jst.go.jp/article/csf1975/18/2/18_2_111/_article
29. Chen EH, Olson EN. Unveiling the mechanisms of cell-cell fusion. Science (New York, N.Y.). 2005 Apr 15 [accessed 2021 May 3]. https://pubmed.ncbi.nlm.nih.gov/15831748/
30. Bastiaans E, Debets AJM, Aanen DK. Experimental demonstration of the benefits of somatic fusion and the consequences for allorecognition. Evolution; international journal of organic evolution. 2015 Mar 30 [accessed 2021 May 3]. https://pubmed.ncbi.nlm.nih.gov/25688421/
31. Schaap P, Barrantes I, Minx P, Sasaki N, Anderson RW, Bénard M, Biggar KK, Buchler NE, Bundschuh R, Chen X, et al. Physarum polycephalum Genome Reveals Extensive Use of Prokaryotic Two-Component and Metazoan-Type Tyrosine Kinase Signaling. OUP Academic. 2015 Nov 27 [accessed 2021 May 3]. https://academic.oup.com/gbe/article/8/1/109/2574153

32. Walter P, Hoffman X-K, Ebeling B, Haas M, Marwan W. Switch-like reprogramming of gene expression after fusion of multinucleate plasmodial cells of two Physarum polycephalum sporulation mutants. Biochemical and biophysical research communications. 2013 May 24 [accessed 2021 May 3]. https://pubmed.ncbi.nlm.nih.gov/23618852/
33. Dussutour A, Vogel D. Direct transfer of learned behaviour via cell fusion in non-neural organisms. Proceedings of the Royal Society B: Biological Sciences. 2016 Dec 28 [accessed 2021 Mar 15]. https://royalsocietypublishing.org/doi/10.1098/rspb.2016.2382
34. Boussard A, Delescluse J, Perez-Escudero A, Dussutour A. Memory inception and preservation in slime moulds: the quest for a common mechanism. Philosophical Transactions of the Royal Society B. 2019 Apr 22 [accessed 2021 May 3]. https://royalsocietypublishing.org/doi/10.1098/rstb.2018.0368
35. Moskvitch K. Slime Molds Remember-But Do They Learn? Wired. 2018 Jul 14 [accessed 2021 Apr 14]. https://www.wired.com/story/slime-molds-rememberbut-do-they-learn/

OTHER REFERENCED MATERIAL:

36. Bonner JT. Brainless behavior: a myxomycete chooses a balanced diet. Proceedings of the National Academy of Sciences of the United States of America. 2010 [accessed 2021 Apr 11];107(12):5267–5268. https://www.pnas.org/content/107/12/5267
37. Cornell University College of Agriculture and Life Sciences. Slime Molds: Myxomycetes. Cornell.edu. 1999 Aug [accessed 2021 Apr 11]. http://plantclinic.cornell.edu/factsheets/slimemolds.pdf
38. Anderson OR. Fine structure observations of phagotrophic activity by plasmodia of Physarum polycephalum. The journal of eukaryotic microbiology. 1993;40(1):67–71. https://pubmed.ncbi.nlm.nih.gov/8457802/
39. Mycetozoa. ScienceDirect. [accessed 2021 Apr 11]. https://www.sciencedirect.com/topics/immunology-and-microbiology/mycetozoa
40. Adamatzky A. Advances in Physarum machines: Sensing and computing with slime mould. 1st ed. Adamatzky A, editor. Basel, Switzerland: Springer International Publishing; 2016. https://play.google.com/store/books/details?id=iPBeCwAAQBAJ
41. Reid CR, Latty T. Collective behaviour and swarm intelligence in slime moulds. FEMS microbiology reviews. 2016 [accessed 2021 Apr 11];40(6):798–806. https://academic.oup.com/femsre/article/40/6/798/2400841
42. Feltman R. What has hundreds of sexes and excels at math? This is Slime Molds 101. Popsci.com. 2019 Oct 17 [accessed 2021 Apr 11]. https://www.popsci.com/the-blob-slime-mold/

43. Bailey J, Anderson RW, Dee J. Cellular events during sexual development from amoeba to plasmodium in the slime mould Physarum polycephalum. Journal of general microbiology. 1990;136(4):739–751. https://pubmed.ncbi.nlm.nih.gov/2398347/
44. Slime mould facts. Warwick.ac.uk. [accessed 2021 Apr 11]. https://warwick.ac.uk/fac/sci/lifesci/outreach/slimemold/facts
45. Yong E. The brainless slime that can learn by fusing. Atlantic monthly (Boston, Mass.: 1993). 2016 Dec 21 [accessed 2021 Apr 11]. https://www.theatlantic.com/science/archive/2016/12/the-brainless-slime-that-can-learn-by-fusing/511295
46. Slime molds remember — but do they learn? Quantamagazine.org. [accessed 2021 Apr 11]. https://www.quantamagazine.org/slime-molds-remember-but-do-they-learn-20180709/