Histological Analysis of the Decision Making Process of Slime Mold, *Physarum polycephalum*

Maci Matanich, Gregory F. Weber

Department of Biology, University of Indianapolis, IN, USA 46227

Introduction

The slime mold, *Physarum polycephalum*, is a single-celled organism that has the ability to make decisions and solve problems without possessing a brain or a central nervous system. Rhythmic contractions allow slime mold to be motile and transport large volumes of its biomass through pumping of cytoplasm, a process known as cytoplasmic streaming (Wohlfarth-Bottermann, 1979). Using cytoplasmic streaming, slime mold can search for the best environmental conditions, including nutritional sources, weighing and balancing available options, then opting for the most suitable condition. When the slime mold is challenged with two paths leading to different food qualities, the so-called two-armed bandit problem, the slime mold reliably favors the better food source (Reid et al., 2016). Slime mold is photosensitive; however, it favors a light exposed food source if the quality of the food is high enough (Latty & Beekman, 2010). Even though a slime mold is a single cell, by differentially distributing its cytoplasm and other subcellular components, it exhibits many collective behaviors seen at the multicellular and population scales of biology. Slime molds can be studied to get a better understanding of how non-cognitive processes and behavior works

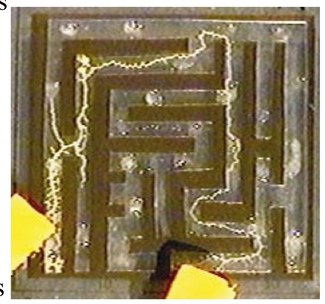


Figure 1: Slime molds have the ability to navigate through a maze to a food source and then refine the cellular network to the shortest, most efficient length possible (Nakagaki et al., 2000).

Structure of *Physarum polycephalum*

Figure 2: *Physarum. polycephalum plasmodium cultured on agar substrate.*

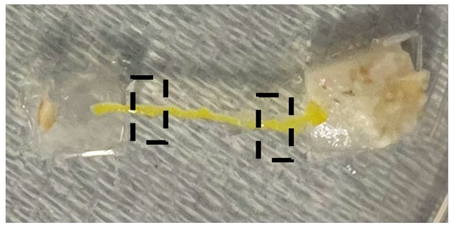
Slime mold is a large single cell that is visible by the naked eye in its plasmodial form. Slime molds are made up of a large tubular network through which protoplasm flows as a result of cytoplasmic streaming (Kamiya, 1981). This is what allows the slime molds to move and adapt the formation of the tubular network. The ability to move and alter the shape of this network is fundamental to the seemingly 'intelligent' behavior exhibited (Nakagaki & Guy, 2008). Slime molds are multinucleated, developing this coenocytic character through repeated rounds of the cell cycle and mitosis without cytokinesis (Howard, 1932). Externally visible asymmetries in tubule diameter develop in the *P. polycephalum* tubule as it moves its cellular mass toward a preferred food source (Reid et al., 2016; Ray et al., 2019). However, it is unknown whether and in what ways the subcellular organization of slime mold changes when it is detecting and deciding the best quality food.

Methods

Physarum polycephalum was cultured in a variety of conditions to promote growth of the biomass and tubule formation. Cultures were grown on either 1% non-nutrient agar with 10% w/v oats, 0.6% non-nutrient agar and 3-4 piles of oats dispersed on the plate, or water cultures (Fig. 3).

Figure 4 (right): For 'decision-making', tubules of *P. polycephalum* were placed between two food sources (10% w/v oat agar block on the right and 1% w/v oat agar block on the left). Dashed boxes indicate regions that would be cut for sampling after decision-making was complete (~2 hours).

Figure 3 (left): *P. polycephalum* was placed on a rubber block in a pool of water about 4cm from another block with 10% w/v oats agar to direct tubule formation. Using either water cultures or agar plate cultures, tubules were collected from the networks and placed into decision-making scenarios.



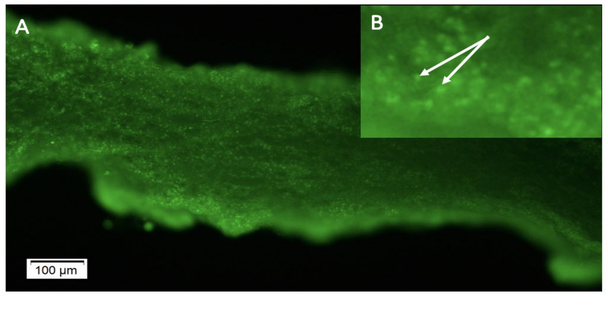
Samples were cut from each tubule end, near the high-quality food source and near the no/low-quality food source and fixed using Trumps fixative for 1-2 weeks. They were washed in 1% MBS 3 times for 10 min each. SYBR Green was used for staining and samples were examined using fluorescence microscopy. Pictures of 3 regions along the tubule were taken. Five random 100x100 µm regions were counted for nuclei.

Results

Table 1: Number of nuclei per 100x100 µm region in sampled tissues from 4 independent tubule decision-making scenarios.

	Tubule 1		Tubule 2		Tubule 3		Tubule 4	
Food Quality	0%	10%	0%	10%	1%	10%	1%	10%
Region 1	22	50	66	63	47	42	22	53
Region 2	60	29	31	26	50	50	26	30
Region 3	40	47	50	40	26	27	38	54
Region 4	41	47	32	37	19	24	42	19
Region 5	44	55	29	35	38	41	35	30
Average	41.40	45.60	41.60	40.20	36.00	36.80	32.60	37.20
Standard deviation	13.52	9.84	16.04	13.77	13.32	10.94	8.35	15.55
Standard error	6.05	4.40	7.17	6.16	5.96	4.89	3.74	6.95

Figure 5: Fluorescence micrograph of a portion of a sample near a high-quality food source (10% w/v oats). Inset in (B) shows enlarged image displaying prominent nuclei (arrows).



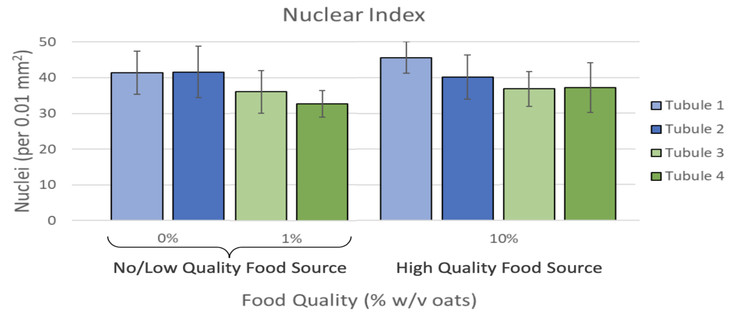
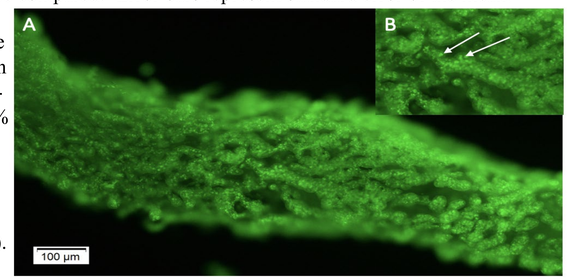


Figure 6: The average number of nuclei among five different regions within a sample were compared to the contralateral end of the same tubule. Each tubule is color-coded so contralateral sides can be compared. Error bars represent standard error of the mean.

Figure 7: Fluorescence micrograph of a portion of a sample near a low-quality food source (1% w/v oats). Inset in (B) shows enlarged image showing nuclei within intracellular compartments (arrows).



Conclusions

- There are is no difference in nuclear densities near high quality or low quality food sources during the decision-making process
- When given two different food sources, slime molds reliably favored the more nutrient dense food source
- The abundance and distribution of other cellular organelles in response to foraging decision making remains an unanswered question

Acknowledgements

We would like to thank the Department of Biology and the Ron and Laura Strain Honors College for their support throughout the project.

References

1. Wohlfarth-Bottermann K.E., (1979). Oscillatory contraction activity in Physarum. *Journal of Biology*, *81*(1), 15-32. https://doi.org/10.1242/jeb.81.1.15.
2. Reid, C. R., MacDonald, H., Mann, R. P., Marshall, J. A., Latty, T., & Garnier, S. (2016). Decision-making without a brain: How an amoeboid organism solves the two-armed bandit. *Journal of The Royal Society* Interface, 13(119), 20160030. https://doi.org/10.1098/rsif.2016.0030
3. Nakagaki, T., Yamada, H., & Tóth, Á. (2000). Maze-solving by an amoeboid organism. *Nature, 407*(6803), 470–470. https://doi.org/10.1038/35035159
4. Latty, T., & Beekman, M. (2010). Food Quality and the risk of light exposure affect patch-choice decisions in the slime mold *Physarum polycephalum*. *Ecology*, *91*(1), 22–27. https://doi.org/10.1890/09-0358.1
5. Kamiya, N. (1981). Physical and chemical basis of cytoplasmic streaming. *Annual Review of Plant Physiology, 32*(1), 205–236. https://doi.org/10.1146/annurev.pp.32.060181.001225
6. Nakagaki, T., & Guy, R. D. (2008). Intelligent behaviors of amoeboid movement based on complex dynamics of soft matter. *Soft Matter, 4*(1), 57–67. https://doi.org/10.1039/b706317
7. Howard, F. (1932). Nuclear Division in Plasmodia of Physarum. *Annals of Botany. 46*(3) 461-477. https://doi.org/10.1093/oxfordjournals.aob.a090331
8. Ray, S. K., Valentini, G., Shah, P., Haque, A., Reid, C. R., Weber, G. F., & Garnier, S. (2019). Information transfer during food choice in the slime mold *Physarum polycephalum. Frontiers in Ecology and Evolution, 7.* https://doi.org/10.3389/fevo.2019.00067