EFFECT OF THE PSYCHEDELIC DRUG DOI ON SYNAPTIC PLASTICITY IN THE NEMATODE *C. ELEGANS*

by

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A THESIS

Presented to the Department of Neuroscience
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

May 2025

An Abstract of the Thesis of

Kyle Stupfel for the degree of Bachelor of Science
in the Department of Neuroscience to be taken June 2025

Title: Effect of the Psychedelic Drug Doi on Synaptic Plasticity in the Nematode *C. Elegans*

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Psychedelic drugs have shown promising potential for treating mental health disorders. Of particular interest is the ability of psychedelic drugs to induce synaptic plasticity, a phenomenon thought to underlie their long-term effects. Research into how psychedelic drugs affect synaptic plasticity would benefit from testing on a genetically tractable animal. The aim of this project is to establish *C. elegans* as a model for studying the effects of psychedelic drugs on synaptic plasticity. We studied the sexually dimorphic synaptic pruning between two neurons, PHB and AVA, in *C. elegans*. We observed a lower number of synaptic puncta in males than in hermaphrodites. Contrary to previously published results, we did not observe a change in sex-specific number of synaptic puncta in response to starvation, and we did observe an increase in synaptic puncta in males in response to serotonin. Experiments with the psychedelic drug 2,5-dimethoxy-4-iodoamphetamine (DOI) led to inconclusive results. Further work is needed to explore the potential effects of psychedelic drugs on synaptic plasticity in *C. elegans.*

Acknowledgements

I would like to express my deepest gratitude to my mentor Dr. Serge Faumont, for his continued guidance throughout my research journey and especially supporting me in finishing my thesis. His knowledge and experience were monumental for helping me tackle tough problems and his consistent support was instrumental for keeping me on time to finish such a big task.

I also am very grateful to Dr. Shawn Lockery, my PI and mentor, who gave me the opportunity to study in his lab. He was a key resource for me in approaching not only my research but also in developing as a scholar and an academic.

To Dr. Nicole Dudukovic, thank you for always being available for support and advice in approaching my thesis and for being so patient throughout the process.

To my peers and friends here in Eugene, I would like to thank you all for your amazing support and love and especially for being so understanding about my hermit-like behavior while working on this project.

To my girlfriend who has supported me through all this process and been a key to helping me get through this final stretch. I am very grateful for you and I'm sure you'd be happy to hear that the worms are dancing.

Finally, I would like to thank my family, who have always supported my academic endeavors. I am eternally grateful for your love, support, and care during this process and beyond. Thank you for all that you do and all that you continue to do.

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Background/Introduction

History of psychedelics

Psychedelic drugs are well-known for their ability to alter perception, mood, and cognitive processes. These drugs include lysergic acid diethylamide (LSD) and psilocybin (magic mushrooms), as well as less familiar drugs like dimethyltryptamine (DMT), 2,5-dimethoxy-4-iodoamphetamine (DOI), and mescaline. The use of psychedelics and their ability to alter cognition dates back thousands of years, with multiple ancient cultures in South America using mind-altering drugs, such as mescaline from peyote plants (George et al., 2022). These drugs were primarily used for ritual and healing practices associated with shamanism, and some cultures still perform these practices today (George et al., 2022). More recently, there was a renewed interest in psychedelic drugs after Albert Hoffman's synthesis of LSD in the 1940s (Kyzar et al., 2017). That resurgence led to psychologists and psychiatrists researching LSD and other psychedelics for their benefits in psychotherapy for a variety of mental disorders (Carhart-Harris & Goodwin, 2017). Along with this resurgence came an increased popularity of psychedelics as recreational drugs. Subsequently, the drugs were then labeled as dangerous and banned by the Controlled Substances Act in 1970 (Belouin & Henningfield, 2018).

Research on psychedelic drugs has ramped up again since the turn of the 21st century, with clinical evidence emerging that the effects of psychedelics, in particular psilocybin, have therapeutic potential. Promising results have been obtained for the treatment of posttraumatic stress disorder, treatment resistant depression, and substance use disorder using psilocybin (Carhart-Harris et al., 2018). Legislation has already been enacted in Oregon that legalized the therapeutic use of psilocybin for adults under the Oregon Psilocybin Services Act (Oregon

Health Authority, 2023). However, many hurdles remain in unlocking the therapeutic potential of psychedelics. In particular, we still lack an understanding of the genetic and molecular pathways involved in psychedelic action, and there are few experimental models to test potential new drugs. Our project aims to develop a new model to test the action of psychedelic drugs in a genetically tractable animal.

Psychedelic action

A distinguishing feature of psychedelic drugs is their ability to achieve long-lasting effects even after a single administration. One possible explanation for these long-lasting effects is that psychedelics can act as *psychoplastogens*, a term describing compounds that promote the formation of new dendrites and synapses in both vertebrates and invertebrates (Vargas et al., 2021). Psychedelics have shown the ability to promote both structural and functional neural plasticity in mammals (Vargas et al., 2023) and fruit flies (Ly et al., 2018). These actions are significant, as it is known that some psychiatric diseases are correlated with neuronal atrophy in the cortex. Therefore, drugs that reverse that atrophy, such as psychoplastogens, could potentially provide new treatments for these diseases (Vargas et al., 2023). These psychoplastogenic effects are thought to be mediated by a serotonin receptor known as 5-HT2A (Kwan et al., 2022). However, the cellular and molecular pathways downstream of 5-HT2A remain a subject of ongoing research. Therefore, it would be useful to have an animal model in which we could study these pathways. In this work, we used the invertebrate *C. elegans* as a model.

***C. elegans* as a model organism**

Caenorhabditis elegans or *C. elegans,* part of the class of worms called nematodes, was first popularized as a genetically tractable model by Sydney Brenner in 1974 (Brenner, 1974; Sulston

& Brenner, 1974). This organism is particularly powerful for studying biological mechanisms due to its small size, short life cycle, transparency, ease of cultivation, and breadth of information already available about genetic, cellular, and neuronal pathways. *C. elegans* has two sexes, self-fertilizing hermaphrodites constitute the majority of the population, with a small proportion of males also present. While having only 302 neurons, *C. elegans* still possess synaptic plasticity. Synaptic plasticity refers to changes in the number or strength of synapses between neurons. Because *C. elegans* is transparent, these neurons and synapses can be visualized *in vivo*. In this study, we investigate the synaptic plasticity between the PHB and AVA neurons in *C. elegans.*

AVA and PHB neurons

PHB is a sensory neuron located in the tail of the animal. It innervates the AVA neuron, an interneuron that plays a role in triggering backward locomotion. PHB is activated by harmful chemicals, hyperosmotic solutions, and mechanical stimulation (Zou et al., 2017) and is thought to inhibit activity in AVA, leading to increased forward locomotion. This circuit therefore leads the animal to escape potentially noxious stimuli at its tail.

PHB and AVA both extend neurites that run alongside each other and form a series of synaptic contacts (or puncta). The synaptic connection between PHB and AVA is present in both males and hermaphrodites at the first larval stage of the worm (Oren-Suissa et al., 2016). However, over the course of development, the number of synaptic puncta decreases in males, but not in hermaphrodites. This developmental decrease in synaptic puncta is called synaptic pruning. Synaptic pruning is common in many organisms and is thought to play essential roles during development. For example, in humans, during adolescence, some areas of the brain lose close to 50% of synaptic connections (Spear, 2013).

Adapted from Bayer, E. A., & Hobert, O. (2018)

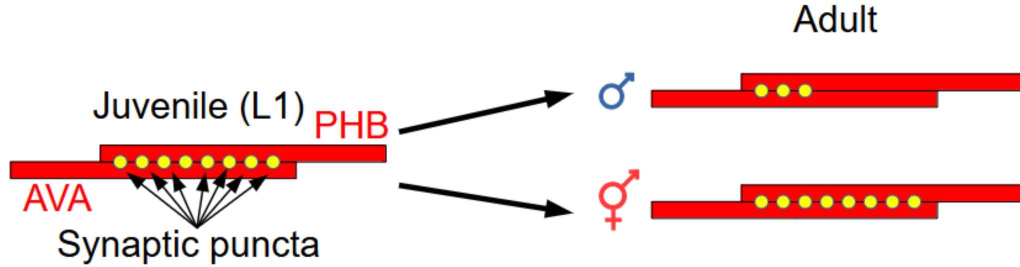

Figure 1. Sex-specific synaptic pruning in AVA and PHB neurons.

At the juvenile stage, male (♂) and hermaphrodite (⚥) worms have the same number of synaptic puncta connecting the AVA and PHB neurons. During development, male worms, but not hermaphrodites, undergo synaptic pruning, resulting in fewer puncta in males as opposed to hermaphrodites.

Synaptic pruning can be modified by experimental manipulations. For example, starvation during development eliminates pruning in males (Liao et al., 2024). This lack of pruning is thought to reflect a lack of serotonin, because food has been shown to increase serotonin release in *C. elegans* (Chao et al., 2004). Conversely, adding back serotonin in starved animals restores pruning (Liao et al., 2024). Therefore, this synaptic pruning in males is dependent on serotonergic signaling. As psychedelic drugs also act through serotonin receptors, we postulate that synaptic pruning could be affected by psychedelic drugs. For instance, psychedelics may act as serotonin substitutes.

Using synaptic pruning as an experimental model to test the effect of psychedelics on *C. elegans* could prove that psychedelics do influence neurons at the level of simple invertebrates. Because not much is known about the genetic and molecular pathways that psychedelics act through, having a simple genetically tractable example of psychoplastogenic behavior would allow for deeper investigation into the pathways responsible.

Experimental Procedures

Nematode Strain, Culture and Solutions

The *C. elegans* strain used in this study was VM6552 *akIs141*[*Prig-3*::*HA*::*glr-1*::*gfp*];*glr-1*(*ky17*), a strain containing a transgene labeling the synapses between AVA and PHB (see below). Nematodes were grown at 20 °C on nematode growth medium plates (NGM; 51.3 mM NaCl, 1.7% agar, 0.25% peptone, 1 mM $CaCl_2$, 12.9 µM cholesterol, 1 mM $MgSO_4$, 25 mM KPO_4, pH 6) seeded with 200 µL of an *E. coli* (OP50) culture as a food. All experiments were performed on young adult hermaphrodites synchronized using a hatch off treatment (see below). Worms were washed and transferred using M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 liter).

Hatch Off Treatment

To make sure animals were all at the same developmental stage, we synchronized our animal population using a hatch off treatment. Adult worms were dissolved in a solution of sodium hypochlorite (NaClO, bleach), and 5M potassium hydroxide (KOH). The eggs inside the animal can survive this treatment as they are enclosed in a tough shell. Collected eggs were transferred to agar plates for 12h to let the eggs hatch and develop to L1 animals (larval stage 1) (Figure 2).

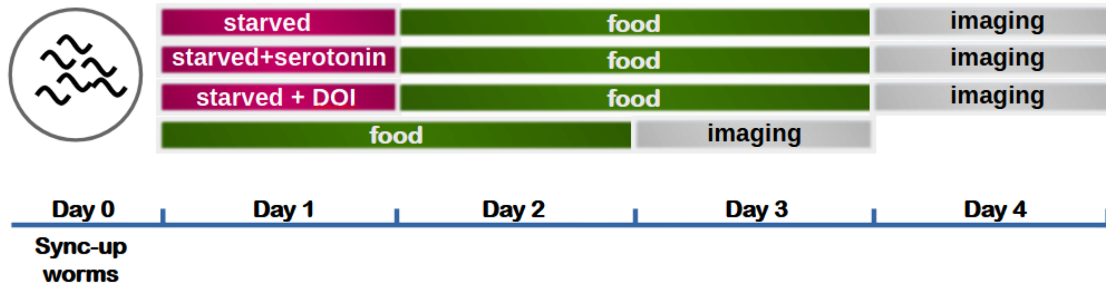

Figure 2. Experimental timeline.

A 5-day experimental timeline showing the timing for drug exposure and imaging for the 4 experimental groups.

Drug Exposure

The worms were then transferred to one of four experimental groups. The first group was the well-fed group and was exposed to food for 48 hours right after hatching before being imaged (Figure 2). The second group, the starved experimental group, was put on a plate with no bacterial lawn for the first 24 hours. The third group was starved on a plate containing 5mM of serotonin. The fourth group was starved on a plate containing 1mM of DOI. Because of the absence of food in the last three groups, the worms do not progress in their development. Therefore, after the 24-hour period of starvation, they were given an additional 24 hours with access to food to allow for their development into adults. (Figure 2)

Fluorescence and GRASP

To visualize the synapses between PHB and AVA each neuron was labeled with a red fluorescent protein (mCherry) and the synapses were labeled with a green fluorescent protein (GFP) using GRASP. GRASP (GFP Reconstitution Across Synaptic Partners), is a technique where two complementary fragments of the GFP are expressed in the two neurons. Where the neurons form synapses, their membranes contact each other, linking these two complementary

fragments and labeling the synapses with GFP (Feinberg et al., 2008). Combining the red and green color channels, the synapses between the two neurons would appear as yellow puncta.

Confocal Microscopy

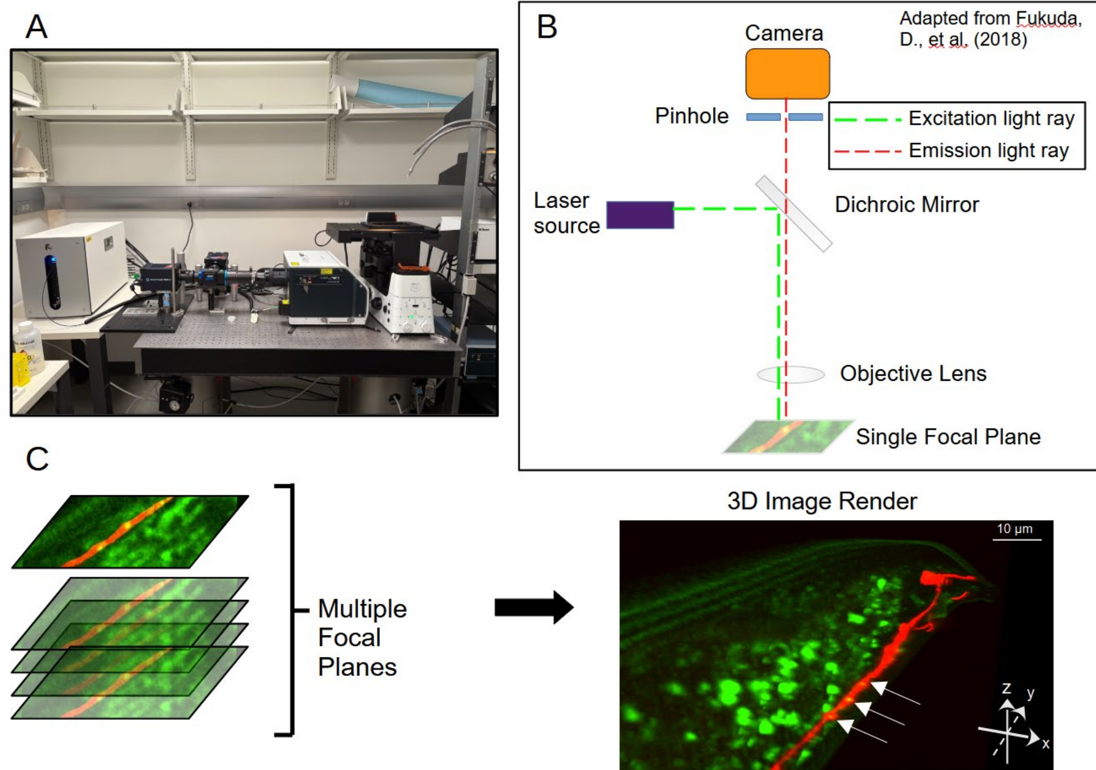

Figure 3. 3-dimensional imaging of synaptic puncta.

A. An image of our confocal microscope.

B. Simplified diagram illustrating the basics of confocal fluorescence microscopy. Adapted from (Fukuda et. al., 2018).

C. Multiple focal planes are combined into a 3-dimensional image. Arrows: synaptic puncta (yellow dots). Red: AVA and PHB neurites. Green: background green fluorescence. Scale bar: 10 microns

We paralyzed the worms using 5mM tetramisole, immobilized them on an agar pad between two glass slides and imaged the preparation on a spinning disk confocal microscope (Figure 3A). In order to image the fluorescent labeled structures, the fluorescent protein was

excited with a laser source (Figure 3B). The resultant excitation light was captured on the camera (Figure 3B). Emission and excitation lights were kept separated using a dichroic mirror which allows for some wavelengths of light to be reflected, like a mirror, while other wavelengths of light pass right through the mirror (Figure 3B). In addition, a defining feature of confocal microscopy is the presence of a pinhole, which restricts light to a plane of focus. The objective (Nikon Plan Fluor; 40x; NA:1.3; Oil Immersion) can move vertically in 0.3 micron increments, capturing single focal planes each time. Stacking these single images allows for a three-dimensional image to be generated (Figure 3C).

Image Deconvolution

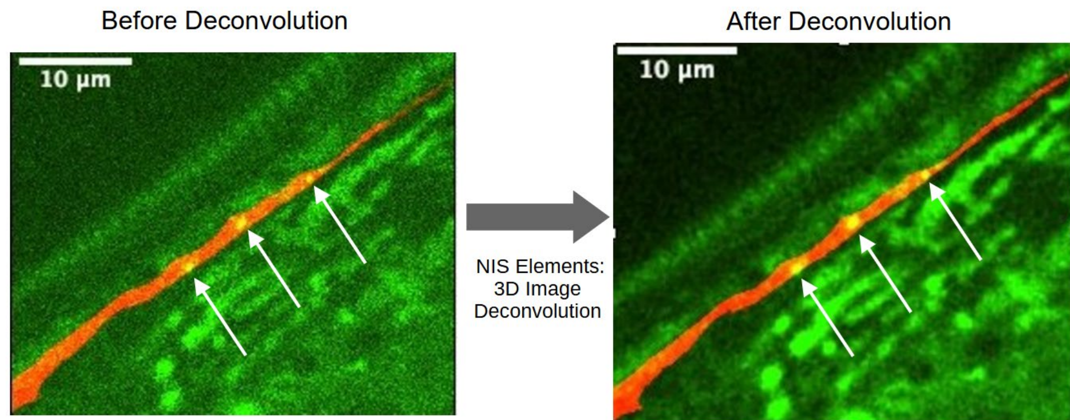

Figure 4. Image deconvolution.

Images before (left) and after (right) deconvolution.

Even with confocal microscopy, light from out-of-focus planes can still 'leak' through to the focal plane leading to blurring of the image (Figure 4). Reassigning this stray light to the correct pixels in the image greatly increases the quality of the image and subsequently our ability to detect synaptic puncta (Figure 4). Deconvolution is a mathematical technique that allows for this reassignment of stray light to its pixels of origin. We applied a deconvolution algorithm

using the confocal microscopes system settings and parameters (NIS Elements: 3D Image Deconvolution) to all our images before further analysis.

WormPsyQi: Semi-automated Image Analysis

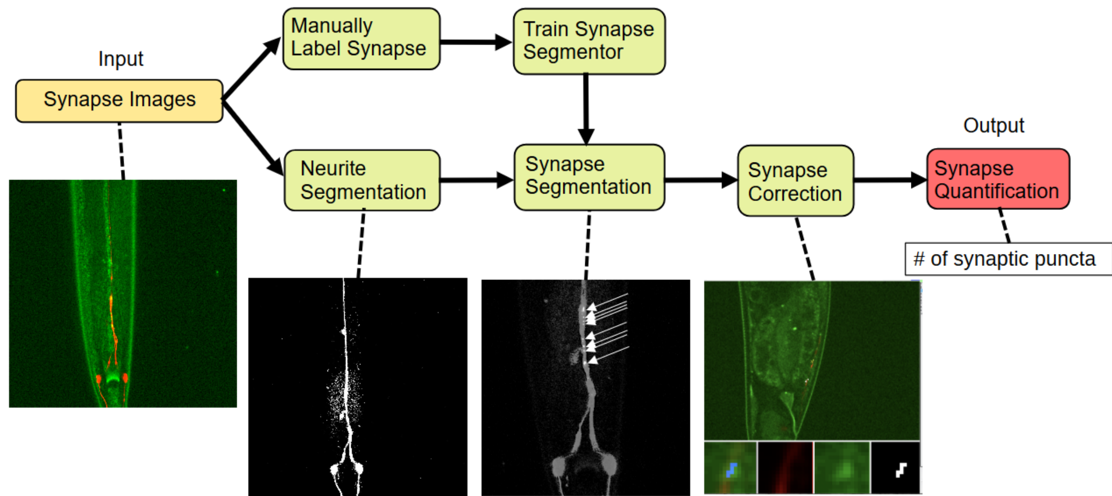

Figure 5. WormPsyQi flowchart for analyzing synaptic puncta.

WormPsyQi is a semi-automated image analysis software created by the Hobert Lab. Using a manually annotated data set and machine learning, the program is trained to recognize synaptic puncta. Following training, all images are segmented, and synaptic puncta are detected and quantified.

To quantify synaptic puncta, we used an image processing software (Fiji) and a trainable image analysis software called WormPsyQi. We began by using those deconvolved images and separated them into a training set and an experimental set. We manually labelled synaptic puncta in the training set (in Fiji) (Figure 5). Those training images were then processed in WormPsyQi to create parameters to recognize puncta. These parameters were then used in the experimental set to detect synaptic puncta: a binary mask of the neurons was first created (neurite segmentation), then a mask of the puncta created (synapse segmentation) (Figure 5). The results

were visually inspected and manually corrected (Figure 5). Once that correction was done, the program automatically quantifies the number of puncta per animal.

Statistical Analysis

Statistical analyses were performed in R version 4.4.2. Two-way ANOVAs were used to assess the interaction between experimental groups and sex. Significance was determined using parametric Welch Two Sample t-tests. This being exploratory data, we were less concerned about false positives than false negatives. For this reason, we chose not to correct our statistical tests for multiple comparison. Outliers were not removed. The threshold for significance was 0.05. In box-and-whisker plots, the box represents 1st and 3rd quartiles, the thick line represents the median, the dotted line represents the mean, and the whiskers indicate the range of data. Details of the statistical analyses, including number of replicates, means, test statistics, p-values, effect sizes, were given in Tables 1 and 2.

Results

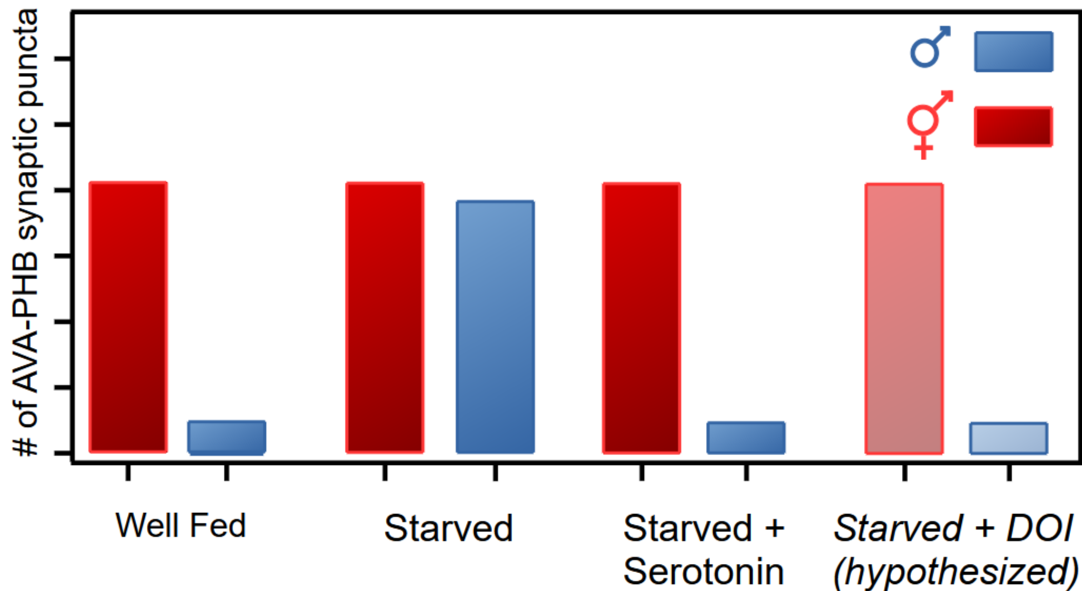

Figure 6. Expected results.

Expected number of synaptic puncta in the four experimental groups. Expected results for the well fed, starved and starved + SER groups are based on Bayer, E. A., & Hobert, O. (2018). Red indicates hermaphrodite, blue indicates male.

Synaptic pruning in males can be modified with environmental conditions like the presence or absence of food as well as the presence of the neurotransmitter serotonin. This synaptic pruning is reflected in a lower number of synaptic puncta in males vs. hermaphrodites. To investigate this phenomenon, we based our experiments on work done by the Hobert Lab (Bayer & Hobert, 2018) . Adult male worms, when well fed in their larval stages, exhibit a significantly lower average number of puncta (Figure 6) in the synapses between neurons AVA and PHB compared to the adult hermaphrodite worms. When starved during the first larval stage however, male worms exhibit the same number of puncta as hermaphrodites (Figure 6). This lack of pruning behavior has been shown to be rescued by the presence of serotonin (Figure 6): when starved during L1 and in the presence of serotonin, the males exhibited a lower puncta count than

hermaphrodites again, similar to that of the well-fed males. We first attempted to replicate these results. We then tested the hypothesis that psychedelic drugs would mimic the effect of serotonin. Specifically, we tested whether the psychedelic drug, 2,5-Dimethoxy-4-iodoamphetamine (DOI) could restore synaptic pruning in starved animals (Figure 6).

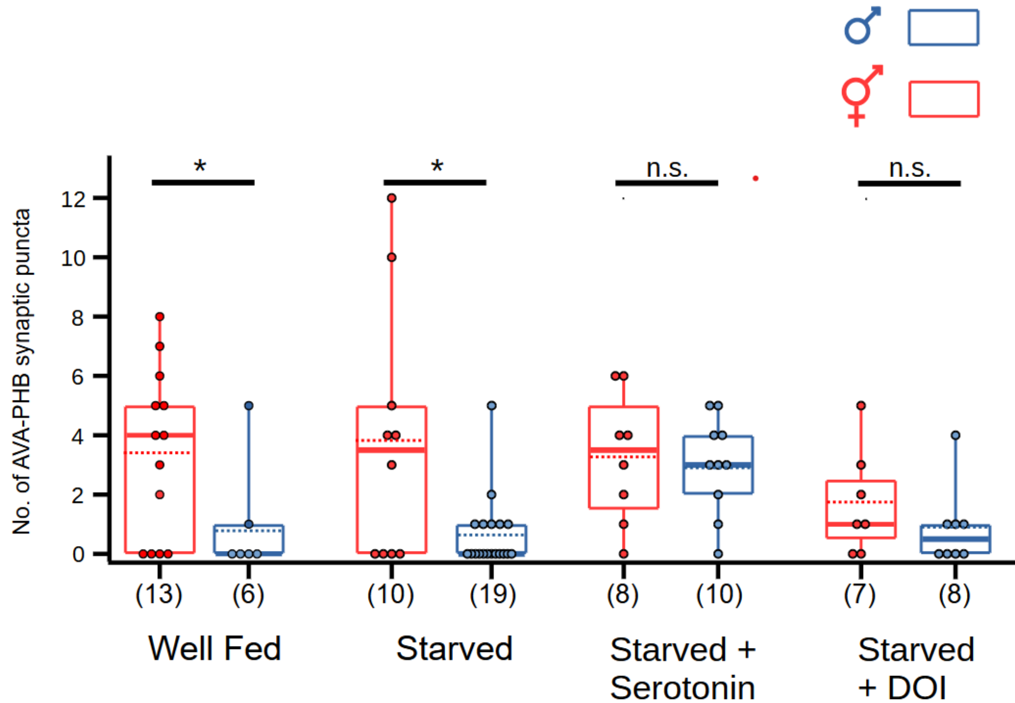

Figure 7. Results of sex-specific synaptic pruning experiments.

Box plots of showing the number of synaptic puncta in hermaphrodites (red) and males (blue) in the four experimental groups. Each dot represents data from a single animal. The lower and upper lines of the boxes indicate the 25th and 75th percentile respectively. The thick line in the box represents the median and the dotted line represents the mean. The whiskers represent the range of data with the lines ending at the minimum and maximum data point. Bars above box plots represent statistics performed by an unpaired *t*-test, $*p < 0.05$, without corrections.

We showed that the male worms with a well-fed environment had significant level of synaptic pruning as demonstrated by lower puncta count (Table 2, Row 2) compared to the hermaphroditic worms. This was consistent with previous work (Figure 6). Surprisingly, starved males still showed a lower number of puncta than hermaphrodites (Table 2, Row 3). Moreover,

males starved in the presence of serotonin now display a puncta count similar to that of hermaphrodites (Table 2, Row 4). These results were directly opposite of the published data and do not demonstrate the expected pruning behavior in response to environmental factors (Figure 6).

We next tested the hypothesis that DOI would mimic the effect of serotonin. An ANOVA comparing starved animals vs. starved + DOI animals failed to detect an effect of DOI but detected an effect of sex, with no interaction (Table 2, Row 5). This suggest that we should see a reduction of puncta count, regardless of experimental group, in males compared to hermaphrodites. Indeed, post-hoc tests showed lower puncta count in the males versus hermaphrodites in the starved group (Table 2, Row 3). Surprisingly, there was no statistical difference in puncta count between sex in the starved + DOI group (Table 2, Row 6). This failure to detect significance was most likely due to low power (Achieved Power: 0.145). In conclusion we were able to observe sexually dimorphic synaptic pruning. However, our results for the effect of starvation and serotonin contradicted previously published data. In addition, our results for the effect of DOI were inconclusive.

Table 1. Summary of data: number of replicates and mean puncta count.

Number of replicates	Mean ± 95% CI
Well Fed ⚥, n=13	count: 3.38 ± 1.47
Well Fed ♂, n=6	count: 1.00 ± 1.46
Starved ⚥, n=10	count: 3.80 ± 2.52
Starved ♂, n=19	count: 0.63 ± 0.53
Starved+Serotonin ⚥, n=8	count: 3.25 ± 1.41
Starved+Serotonin ♂, n=10	count: 3.00 ± 0.96
Starved+DOI ⚥, n=7	count: 1.71 ± 1.23
Starved+DOI ♂, n=8	count: 0.88 ± 0.88

Table 2. Statistical tests

Fig.	Row	Comparison	Test	Statistic	p-value	Signif.
7	1	Effect of experimental group (Well Fed, Starved, Starved+Serotonin) and sex on number of puncta	ANOVA			
			Main effect of Experimental Group	F(2,60) = 1.92	0.15615	
			Main effect of Sex	F(1,60) =10.9	0.00161	**
			Interaction Group × Sex	F(2,60) =1.89	0.15930	
		Post-hoc tests				
	2	Effect of sex on Well Fed worms puncta count	Welch Two Sample t-test	t(13.59) = 2.11	0.0500	*
	3	Effect of sex on Starved worms puncta count	Welch Two Sample t-test	t(9.76) = 2.29	0.0460	*
	4	Effect of sex on Starved+Serotonin worms	Welch Two Sample t-test	t(12.67) = 0.27	0.7920	
	5	Effect of experimental group and sex on number of puncta on Starved and Starved+DOI	ANOVA			
			Main effect of Experimental Group	F(1,40) = 0.37	0.54702	
			Main effect of Sex	F(1,40) =9.90	0.00312	**
			Interaction Group × Sex	F(1,40) =2.30	0.13720	
		Post-hoc test				
	6	Effect of sex on Starved+DOI worms puncta count	Welch Two Sample t-test	t(11.10) = 1.01	0.3347	
	7	Effect of experimental group and sex on number of puncta on Starved and Starved+Serotonin	ANOVA			
			Main effect of Experimental Group	F(1,43) = 3.69	0.06166	
			Main effect of Sex	F(1,43) =7.50	0.00896	**
			Interaction Group × Sex	F(1,43) =3.89	0.05512	

Statistical significance is performed by an unpaired t-test, *$p < 0.05$, **$p < 0.01$ before corrections.

Discussion

In this study we investigated a sex-specific, serotonin-dependent synaptic pruning mechanism in *C. elegans* as a potential model for psychedelic-induced synaptic plasticity. We were able to observe synaptic pruning in males but were unable to replicate previous results showing that synaptic pruning is influenced by starvation or serotonin. As for the effect of the psychedelic drug DOI on synaptic pruning, our results are inconclusive.

Hobert et al. (2018) showed that the effect of starvation on male *C. elegans* is a lack of synaptic pruning in the PHB-AVA neurons, which leads to a puncta count in males similar to that in hermaphrodites. This effect is also shown to be reversed by serotonin, leading to a puncta count in males that is significantly lower than hermaphrodites. We found that the effect of starvation in males was actually a significantly lower puncta count compared to hermaphrodites and the presence of serotonin leads to a puncta count in males similar to the puncta count in hermaphrodites.

The difference between the previously published data and our work is possibly the result of changes in experimental methodology, such as small differences in hatch-off protocol. Previous work hatched the worms in liquid, whereas we hatched the worms on a plate, because the liquid protocol resulted in excessive mortality.

In addition, synaptic puncta were manually labeled in past work, whereas we relied on a trainable software, WormPsyQi. There were also no clear published criteria for what was labeled as puncta. This could lead to potential bias when manually labelling puncta. We were able to eliminate potential experimenter bias by relying on trainable software to identify puncta.

Finally, the differences between previous work and our own may simply reflect the high variability in our data. We obtained only a minimum of 7-8 replicates per group, compared to 12-

16 worms per experimental group in previous work (Bayer & Hobert, 2018). The high variability combined with the low number of replicates limits the potential conclusions that can be drawn from our data.

Exposure to the psychedelic DOI led to ambiguous results with a lack of effect of DOI when compared to the starved control group, but also no difference in the number of puncta between males and hermaphrodites in the DOI-treated group. As noted above, the lack of power of our statistical tests and the fact we were unable to replicate previous work limit the conclusions we can draw from the DOI experiment.

Future work will include increasing the number of replicates to bolster statistical power. We would also like to try similar experiments with different psychedelics that we have seen have more reliable effects on the behavior of *C. elegans*, like LSD. These next steps will allow us to further investigate *C. elegans* ability to act as a model for psychoplastogenic effects. Having a genetically tractable animal model that exhibits a response to psychedelics would allow for a better understanding of the genetic and molecular pathways. Using the *C. elegans* model would also allow for a better experimental model for testing newly developed psychedelics for psychoplastogenic effects.

Bibliography

Authority, O. H. (2023, April 12). *Psilocybin 101: What to know about Oregon's psilocybin services*. Oregon Health News Blog. https://oregonhealthnews.oregon.gov/psilocybin-101-what-to-know-about-oregons-psilocybin-services/

Bayer, E. A., & Hobert, O. (2018). Past experience shapes sexually dimorphic neuronal wiring through monoaminergic signaling. *Nature*, *561*(7721), 117–121. https://doi.org/10.1038/s41586-018-0452-0

Belouin, S. J., & Henningfield, J. E. (2018). Psychedelics: Where we are now, why we got here, what we must do. *Neuropharmacology*, *142*, 7–19. https://doi.org/10.1016/j.neuropharm.2018.02.018

Brenner, S. (1974). THE GENETICS OF CAENORHABDITIS ELEGANS. *Genetics*, *77*(1), 71–94. https://doi.org/10.1093/genetics/77.1.71

Carhart-Harris, R. L., Bolstridge, M., Day, C. M. J., Rucker, J., Watts, R., Erritzoe, D. E., Kaelen, M., Giribaldi, B., Bloomfield, M., Pilling, S., Rickard, J. A., Forbes, B., Feilding, A., Taylor, D., Curran, H. V., & Nutt, D. J. (2018). Psilocybin with psychological support for treatment-resistant depression: Six-month follow-up. *Psychopharmacology*, *235*(2), 399–408. https://doi.org/10.1007/s00213-017-4771-x

Carhart-Harris, R. L., & Goodwin, G. M. (2017). The Therapeutic Potential of Psychedelic Drugs: Past, Present, and Future. *Neuropsychopharmacology*, *42*(11), 2105–2113. https://doi.org/10.1038/npp.2017.84

Chao, M. Y., Komatsu, H., Fukuto, H. S., Dionne, H. M., & Hart, A. C. (2004). Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(43), 15512–15517. https://doi.org/10.1073/pnas.0403369101

Feinberg, E. H., VanHoven, M. K., Bendesky, A., Wang, G., Fetter, R. D., Shen, K., & Bargmann, C. I. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) Defines Cell Contacts and Synapses in Living Nervous Systems. *Neuron*, *57*(3), 353–363. https://doi.org/10.1016/j.neuron.2007.11.030

George, D. R., Hanson, R., Wilkinson, D., & Garcia-Romeu, A. (2022). Ancient Roots of Today's Emerging Renaissance in Psychedelic Medicine. *Culture, Medicine and Psychiatry*, *46*(4), 890–903. https://doi.org/10.1007/s11013-021-09749-y

Kwan, A. C., Olson, D. E., Preller, K. H., & Roth, B. L. (2022). The neural basis of psychedelic action. *Nature Neuroscience*, *25*(11), 1407–1419. https://doi.org/10.1038/s41593-022-01177-4

Kyzar, E. J., Nichols, C. D., Gainetdinov, R. R., Nichols, D. E., & Kalueff, A. V. (2017). Psychedelic Drugs in Biomedicine. *Trends in Pharmacological Sciences*, *38*(11), 992–1005. https://doi.org/10.1016/j.tips.2017.08.003

Liao, C.-P., Majeed, M., & Hobert, O. (2024). Experience-dependent, sexually dimorphic synaptic connectivity defined by sex-specific cadherin expression. *Science Advances*, *10*(46), eadq9183. https://doi.org/10.1126/sciadv.adq9183

Ly, C., Greb, A. C., Cameron, L. P., Wong, J. M., Barragan, E. V., Wilson, P. C., Burbach, K. F., Zarandi, S. S., Sood, A., Paddy, M. R., Duim, W. C., Dennis, M. Y., McAllister, A. K., Ori-McKenney, K. M., Gray, J. A., & Olson, D. E. (2018). Psychedelics Promote Structural and Functional Neural Plasticity. *Cell Reports*, *23*(11), 3170–3182. https://doi.org/10.1016/j.celrep.2018.05.022

Oren-Suissa, M., Bayer, E. A., & Hobert, O. (2016). Sex-specific pruning of neuronal synapses in Caenorhabditis elegans. *Nature*, *533*, 206–211. https://doi.org/10.1038/nature17977

Spear, L. P. (2013). Adolescent Neurodevelopment. *The Journal of Adolescent Health : Official Publication of the Society for Adolescent Medicine*, *52*(2 0 2), S7-13. https://doi.org/10.1016/j.jadohealth.2012.05.006

Sulston, J. E., & Brenner, S. (1974). THE DNA OF CAENORHABDITIS ELEGANS. *Genetics*, *77*(1), 95–104. https://doi.org/10.1093/genetics/77.1.95

Vargas, M. V., Dunlap, L. E., Dong, C., Carter, S. J., Tombari, R. J., Jami, S. A., Cameron, L. P., Patel, S. D., Hennessey, J. J., Saeger, H. N., McCorvy, J. D., Gray, J. A., Tian, L., & Olson, D. E. (2023). Psychedelics promote neuroplasticity through the activation of intracellular 5-HT2A receptors. *Science*, *379*(6633), 700–706. https://doi.org/10.1126/science.adf0435

Vargas, M. V., Meyer, R., Avanes, A. A., Rus, M., & Olson, D. E. (2021). Psychedelics and Other Psychoplastogens for Treating Mental Illness. *Frontiers in Psychiatry*, *12*, 727117. https://doi.org/10.3389/fpsyt.2021.727117

Zou, W., Cheng, H., Li, S., Yue, X., Xue, Y., Chen, S., & Kang, L. (2017). Polymodal Responses in C. elegans Phasmid Neurons Rely on Multiple Intracellular and Intercellular Signaling Pathways. *Scientific Reports*, *7*, 42295. https://doi.org/10.1038/srep42295