Demystifying Psilocybin - a science-based framework for understanding its actions

This is the script, including references cited, for the seminar presentation that can be watched on YouTube at https://youtu.be/qBM5BX8WSyc . The seminar was delivered on June 10, 2021. It was developed and presented by the 16 members of the Oregon State University Spring 2021 Biochemistry and Biophysics Capstone class: Kyle Axt, Michael Dickens, Felisha Imholt, Audrey Korte, Jac Longstreth, Duncan MacMurchy, Jacob North, Seth Pinckney, Sanjay Ramprasad, Danielle Sanchez, Juno Valerio, Cat Vesely, Monica Vidal-Franco, Daniel Whittle, Jun Yang and instructor Andy Karplus.*

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Script

Intro-1. Welcome. Welcome! I'm really pleased to introduce our Biochemistry and Biophysics Capstone class who, given the enactment of Oregon Measure 109, took on the task of digging through scientific papers to create a non-controversial science-based perspective on psilocybin and the molecular mechanisms behind its actions. We discovered many diverse studies are relevant, so ... rather than going deep into a few studies, we've sought to construct a broad overview of key topics in hopes of giving both scientists and the public a window into the fascinating science of psilocybin along with pointers to the literature to allow follow up on topics of interest.

Intro-2. Measure 109. Oregon Measure 109 passed in November 2020.[1] This legalized the research, development, and use of psilocybin by licensed providers as a therapeutic for mental illnesses such as anxiety & depression. This may be news to you, but out of the 50 states, Oregon, at 22.6%, has the highest prevalence of mental illnesses in adults.[2] No licenses are yet being created, as an initial two year development period – through January 2023 – was created so that the Oregon Health Authority and a 14-16 member advisory board could create the rules and regulations needed to improve the overall safety of the implementation of psilocybin therapy. Finally, the measure had up-front costs covered by the general fund and a psilocybin tax covering the long-term costs.

Intro-3. Outline. We have built today's presentation around 10 main take-home messages: 4 on biochemical and historical background, 2 on mechanisms behind the psychedelic experience, 1 on clinical trials of therapeutic potential, and 3 on probing mechanisms related to possible therapeutic effects. Let's start our story now with the important molecule known as serotonin, as that is what psilocybin mimics.

1-1. Serotonin discovery. Two teams working on different biological systems independently discovered serotonin. In the 1930s, while probing the skin and intestinal tracts of rabbits, mollusks, and frogs for amines that could cause smooth muscle contraction, Dr. Vittorio Erspamer discovered a substance in cells lining the gut.[3] He named this substance "enteramine" after its source in the GI tract. During the 1940s, Maurice Rapport, Arda Green, and Irvine Page were studying hypertension and searching for constricting factors in blood.[4] They discovered that such a substance was produced when blood coagulated, and they named it "serotonin". Once the

structures of these two substances were elucidated, they were found to be the same substance, now called serotonin or 5-hydroxytryptamine, often abbreviated 5-HT.[5]

Betty Twarog was the first to find serotonin in the brain. She sought a neurotransmitter that would counteract acetylcholine and cause muscle relaxation.[6] After reading Dr. Erspamer's work, she identified serotonin as the unknown neurotransmitter and went on to develop sensitive tests for it and prove its presence in the brain.[7] In a funny twist of fate, LSD actually played an early role advancing neuroscience. D.W. Wooley noticed that the structure of LSD was similar to serotonin, and noting the powerful effects of LSD on the mind, his 1954 paper makes three prescient proposals – that (*1) serotonin plays a role in mental processes; (2) serotonin deficiencies may contribute to mental disorders; and (3) serotonin or analogs may alleviate mental health disorders.*[7] So from early on there have been connections between serotonin and psychedelics and the potential value of psychedelics for treating mental illness.

So how does serotonin do its work?

1-2. Serotonin is ancient. Serotonin is found in single cell eukaryotes and virtually all multicellular organisms, leading to the conclusion it is quite ancient, predating even the dopamine and adrenaline systems. Serotonin has amazingly broad functions in our bodies, with impacts including mood, appetite, sleep, stress response, sexuality and even the formation of memories. Serotonin works through binding to transmembrane protein receptors that trigger specific intracellular responses. Of the 14 known human serotonin – or 5-HT – receptors one, type 3, is an ion channel, colored green here because by allowing cations to enter the cell it stimulates nerves to fire. The others are so-called G-protein coupled receptors, or GPCRs, which trigger many complex signaling events. Among the 6 subgroups, some excite nerves to fire (colored green) and some inhibit nerve firing (colored red).[8] GPCRs are a prolific receptor family, with approx. 900 identified in humans. They are hugely important, with functions including responding to light allowing us to see, and odorants allowing us to smell, and neurotransmitters like adrenaline, dopamine, glutamate, opioids and of course serotonin.[9] Their importance is perhaps made even more clear by noting that nearly a third of all FDA approved small molecule drugs target a GPCR receptor![10]

1-3. Serotonin as neurotransmitter. 95% of our serotonin is made in the intestines, with the raphe nucleus in the brain stem as another main site of synthesis and distribution.[11] Overall, about 90-95% is found in the intestines, 8% in platelets, and just 1-2% in the central nervous system.[12] As a neurotransmitter signaling between neurons, serotonin is stowed in vesicles by the specialized monoamine transporter, VMAT2. Upon excitation**,** the stored serotonin is released into the synapse, where its interaction with receptors stimulates other neurons. The serotonin transporter SERT then recovers the serotonin, and the enzyme monoamine oxidase works inside and outside the cell to convert serotonin to an inactive product.[13]

1-4. Serotonin and mental health. As is widely known, imbalances in serotonin levels can be linked to mood disorders such as depression, schizophrenia and aggression.[14] SSRIs – blocking serotonin uptake[15] – and MAO inhibitors – blocking serotonin degradation[16] – both raise serotonin levels and are clinically useful for treating depression and anxiety. This is all relevant to psilocybin, because psilocin - the active form of psilocybin, is a serotonin analog that is transported by SERT and degraded by MAO.

1-5. Serotonin in insects. Also because of relevance to psilocybin, we note here that in insects, serotonin regulates sleep, swarming behavior, learning, and notably, appetite. As one example, in one species of ant, the volume of sucrose ingested as well as the intake rate were lowered with

serotonin injections, and furthermore, as seen in this other image, this effect was dose dependent.[17] Capitalizing on this relationship, serotonin-mimicking insecticides such as pymetrozine have been developed that inhibit feeding in sucking insects like aphids and white flies.[18]

1-6. Take-home #1. Serotonin is an ancient and widespread signaling molecule influencing myriad behaviors ...with 14 receptors in humans, some excitatory and some inhibitory

So that's serotonin, but what about psilocybin?

2-1. Natural Products and Medicine. Living organisms produce a huge variety of organic compounds known as "secondary metabolites". Psilocybin is one example. These are not required for life, but often provide an evolutionary advantage to the organism.[19,20] Many are useful for medicinal purposes, and in fact over 50% of FDA approved drugs are natural products or molecules inspired by natural products.[21] For instance, the well-known anti-tumor medication TAXOL was obtained from the bark of the Pacific yew tree.[22] However, not all natural products are beneficial. For instance, many mushroom species produce the natural product muscarine. The consumption of which results in sweating, lacrimation, bradycardia, and even death when consumed by animals.[23]

Natural products in medicine are often modified to eliminate such effects. Despite the dangerous effects of natural muscarine, its derivatives have been used for the treatment of inflammatory uveitis and glaucoma. The process of making derivatives of natural products and other drugs leads to improving their efficacy and decreasing unwanted side effects. This is how most drugs are developed. Notice in the pie chart that many more natural product derivatives are used as drugs than unaltered natural products. That is because natural products have not been optimized for their use in human medicine!

2-2. Modifying Natural Products Improves Efficacy. One simple example of the process is penicillin-based antibiotics.[24] The natural product penicillin was improved to ampicillin – which was a broader spectrum antibiotic – and then to amoxicillin which was faster acting and stronger. The value of natural products has inspired numerous screening programs, for instance, the National Cancer Institute has programs to discover more natural product antitumor agents, and the National Institute for Mental Health has created the Psychoactive Drug Screening Program or PDSP that has already screened over 50,000 compounds in search of new compounds that target neurotransmitter receptors and may be useful for treating mental health disorders.[22]

Getting back to psilocybin, how do the mushrooms make it?

2-4. Biosynthesis: Making "Magic." *(Michael) 1 min 3 sec* Psilocybin synthesis requires only 4 enzymes... The first step is the removal of carbon dioxide… Next an OH-group is added to the 4 position of the ring structure. Then a phosphate group is added on... Lastly to form psilocybin two CH3-groups are added to the amine nitrogen.[25,26]

But psilocybin is easily dephosphorylated, resulting in psilocin, that is actually the bioactive form, but the phosphate-adding enzyme from before ensures psilocin concentrations stay low by re-phosphorylating it. When exposed to oxygen, psilocin reacts with other psilocins forming larger molecules which explains the striking blue color that appears when psilocybin-producing mushrooms are damaged.[25]

Two other interesting tidbits are that, baeocystin can also be dephosphorylated forming a second psychoactive compound ... and the fungi also produce beta carbolines which can inhibit MAO to enhance the impact of psilocin.[27]

Now why do mushrooms go through all this effort to make psilocybin?

2-5. Why do fungi make psilocybin? The advantage psilocybin provides remains unknown, with one idea being it provides protection from insects, by messing with their serotonin signaling, for instance decreasing their appetite, as noted earlier. Psilocybin's retention in wood-decay and dung decay fungi supports this idea since both environments are rich in insects, such as termites, that compete for the food source and/or that eat fungi.[28] Additional evidence is that among mushrooms that mostly make the toxin muscarine as protection from insects, a few groups make psilocybin, and notably as seen in this figure, a loss of muscarine production always accompanies the gain of psilocybin production as if psilocybin is performing the same function.[23]

2-6. Some commonly known psychedelics. *(Cat)* Besides psilocybin, other natural products also have psychedelic effects. Classic Psychedelics, all acting mainly through the 5-HT_{2A} receptor include Psilocybin, Mescaline found in peyote cactus, DMT found in Ayahuasca (ˌīəˈwäskə) plants,[29] Ibogaine from the Iboga tree[30] and synthetic LSD[29] and designer drugs like 25CN-NBOH.[31] ..,. Other psychedelics – such as MDMA, ketamine and Salvinorin A – have different mechanisms.[32,33] Importantly, studies of one compound are often informative about the others in that same group (like the "classic" psychedelics), but NOT about those in other groups. So, the take home for this section is

2.6. Take-home #2. Psilocybin is not "magic" but one of thousands of bioactive natural products that may be medically useful itself and also potentially useful as a lead compound that can be optimized

Let's turn and look briefly at some history of psilocybin use and research into psychedelics

3-1. Historic Use of Magic Mushrooms. The earliest evidence of humans using psilocybin is mushrooms depicted in cave paintings at Villar del Humo in Spain that are 3,500 to 8,000 years old, although it is unclear if they are indeed psychedelic mushrooms. Across the ocean in Mesoamerica, "Mushroom Stones," found in several Central American countries, have been dated back to 500 BCE to 900 CE. Psychedelic mushrooms, known as *teonanacatl* or "God's Flesh" to the Aztecs, were used in rituals described in 1529 by Spanish ethnographer Friar Bernardino de Sahugun. These descriptions inspired R. Gordon Wasson and Roger Heim to travel to Mexico in 1955 in search of psychedelic mushrooms. They identified several species of *Psilocybe* mushrooms and in 1957, sent a sample to Albert Hofmann, who identified psilocybin as the psychedelic compound. By 1960, clinical research had begun.[34,35]

3-2. Controlled Substances Act of 1970 Halted Hallucinogen Research. From 1960-1970 psychedelic research was exploding, with over a thousand papers exploring the therapeutic uses of classic hallucinogens. This research, however, came to an abrupt end with the controlled substances act of 1970. Hallucinogens like psilocybin – with no approved medical use – were placed into schedule 1. These were greatly restricted, requiring federal and state licensing for research, manufacturing, and distribution which were nearly impossible to obtain. There were also heavy penalties for breaking these restrictions, and Schedule 1 drugs suffered a severe decline in research.[36,37] We can see this by comparing publications for the schedule 1 hallucinogen LSD with the schedule 2 opioid morphine. After 1970, publications began to plummet for LSD with little

or no impact on morphine publications**,** LSD going from nearly half as many publications as morphine before 1970 to about 1/10th as many publications since then.

Now you might be wondering how research into psychedelics got restarted.

3-3. The Psychedelic Research Revival. In 1992, both the NIDA and the FDA determined that research into psychedelics should resume. In 1986 and 1993, two non-profit organizations were founded by Rick Doblin and David Nichols, respectively, with the goal of funding research into psychedelics through stage 3 clinical trials at top institutions.[38] This was in contrast to previous clinical research that was often not done in a systematic manner.

MAPS chose to focus on MDMA. The Heffter Institute considered LSD, but its "social baggage" and long duration made it less ideal for clinical trials. Mescaline and MDMA were rejected due to their negative side effects. Psilocybin had little "baggage" and a great safety profile and became their choice.

Today, several universities have centers dedicated to psychedelic research including Johns Hopkins, the Imperial College London, and, as of January 2021, Mount Sinai.

3-4. Mainstream Again: ACS "Dark Classics." Now, research into the neuroscience of psychedelics and their potential use in controlled treatment of disorders such as anxiety, depression, and PTSD seems to be fairly mainstream. Reflecting this, the journal *ACS Chemical Neuroscience* published special "Dark Classics" issues in 2018 and 2020 to pull together in one place information on the properties of many previously stigmatized drugs including psychedelics such as psilocybin.[39–41]

3-4. Take-home #3. Early work on the therapeutic potential of psychedelics was abruptly curtailed in 1970 but is now again mainstream neuropharmacology.

Underpinning the psychedelic experience are G-protein coupled receptors: the amazing molecular machines that allow neurotransmitters to signal elaborate cellular cascades. As our last introductory topic, we'll take a highly simplified look at how GPCRs work.

4-1. Basic GPCR structural features. All G-Protein coupled receptors share the same architecture: seven helices (sequentially named 1 thru 7) are arranged in a barrel shape spanning the phospholipid membrane.[42] Neurotransmitters bind on the extracellular side in a pocket called the "orthosteric binding site", where they make precise interactions that force helices 6, 3, 5, and 7 apart, which opens a binding pocket inside the cell. Specific G-proteins then bind here and become activated, splitting the GDP-bound complex into an alpha unit with GTP bound, plus a beta-gamma unit. Each of these then goes on to send further signals.[43] To discuss how GPCRs work, a clever naming scheme identifies its parts by which helix they are in and their position relative to that helix's most conserved residue, which is assigned the number "50" – as shown here in magenta for each helix. For instance, in the 5-HT_{2A} receptor, residue tryptophan 3-hundred-36 would be "W336 superscript 6.48" meaning it is right about here in helix 6. I chose this residue as an example because a recently-published structure[31] of the serotonin 5-HT_{2A} receptor showed it was a key switch activating this *dramatic* four-helix rearrangement. Although it's normally in the "off" state, if an activating ligand – or "agonist" – binds, it displaces tryptophan 3-hundred-36, which bends helix 6 and opens the pocket. In this diagram – where each dip is a different shape with a particular activity – we summarize the *important* idea that GPCRs are a *mixture* of a few different wiggly shapes rather than being completely on or off. *Two* forms are off, *one* form is turned *on* by *one* type of interaction, and *another* form is turned on by a *different* type

of interaction – where the *mix* of these states depends on the neurotransmitter. Furthermore, even *without* a neurotransmitter, the receptor is not *completely* off, but in this case only *eighty percent* off.

Continuing our simplified summary of GPCRs, let's look more closely at how signals flow from these shape changes.

4-2. Each G-Protein triggers multiple signaling pathways. Shown here are simplistic "off", "on-1" and "on-2" shapes to separately show what each does. The "off" shape is easy, it doesn't do anything. The "on-1" shape, as noted in the previous slide, turns on the G-protein alpha and the beta-gamma units so they can impact the cell by turning on or off certain signaling pathways. As a remarkable feature, each activated G-protein turns itself off after a short time by converting GTP back to GDP. The "on-2" shape allows the GPCR to bind to "arrestin", so-named because it stops the receptor from turning on more G-proteins. This process**,** known as desensitization, can happen within minutes of activation and requires a G-protein receptor kinase (GRK). However, it is now known that in addition to stopping G-protein signaling, arrestin actually triggers additional signaling events and then receptor internalization for recycling or degradation.

Revisiting the shape preference diagram, we can introduce some important terms. First, recall that a resting GPCR might be say have a baseline activity or 10% on for G-protein signaling and 5% on for arrestin signaling. An "antagonist" is a molecule that blocks the binding of other molecules without changing the resting activity. "Agonists' ' turn on G-protein signaling, and transition to arrestin-based signaling, just like the true neurotransmitter, and "partial agonists" turn on some fraction of the full activity. But there is more. Various "biased agonists" differentially favor the on-1 or on-2 shapes and so can lead to huge varieties of signaling outcomes. And finally an inverse agonist stabilizes the "off" shape and so turns off the baseline receptor activity - leading to an opposite outcome from an agonist.[43] You can see why simply considering the affinity of a molecule for a receptor is not the whole story!

So what types of events do the serotonin GPCRs trigger?

4-3. Three G-Protein options for 5-HT signaling. Each 5-HT receptor GPCRs signal through one of three G protein subtypes and we here only take a highly simplified look at what makes some excitatory and some inhibitory for neuronal firing. 5-HT_2 receptors are excitatory and couple to Gq proteins resulting, in addition to other outcomes, in phospholipase C activation that eventually leads to an influx of Ca^{2+} into the cell enhancing excitability. The 5-HT4, 6 and 7 receptors couple to Gs proteins leading to increased cAMP and again in addition to many other things, a net increase in positive ions flowing into the cell promoting neuronal firing. In contrast, the 5-HT1 and 5 receptors couple to Gi/o proteins resulting in a lowering of cAMP-and the opposite effects on ion channels inhibiting neuronal firing.[44]

Now let's get back to looking at how GPCRs responses can differ for various ligands.

4-4. How Ligands Impact GPCR Signaling. The impact of a GPCR ligand is commonly shown in graphs like this where the level of signaling in either the G-protein- or arrestin-specific pathways are plotted against the logarithm of the ligand concentration. Each activity curve will start at the resting or baseline level of activity that occurs in the absence of a ligand. Moving on, the red curve shows the impact of the natural ligand or an agonist binding. The activity generated by these ligands is defined as 100%. A partial agonist, in yellow, produces submaximal activation of receptor signaling even when all receptors are occupied. Inverse agonists, shown in blue block even the resting activity by stabilizing the inactive conformation. Antagonists, shown in purple,

bind the receptor without changing the activity. This means they are fantastic tools for blocking the activity of agonists. Biased ligands more strongly activate one pathway over another, as is illustrated in orange for an arrestin-biased ligand, stimulating arrestin-based signaling much more than G-protein-based signaling.[45]

Here, we illustrate using a couple of psychedelics bound to the 5-HT2A receptor, the kind of subtle differences in binding that can lead to big differences in activity. Shown in yellow is strong agonist, which is the only ligand that displaces the key switch residue, W336, that shifts helix H6. In blue is LSD, an arrestin-biased agonist, and the other molecules are all inverse agonists.[31] Note they all bind basically to the same pocket despite the large differences in the signaling events they will trigger.

4-5. Take-home #4. This leads us to Take-home #4 which is: GPCRs are remarkable shape-shifting molecules that impact cell behavior through a complex dance of G-proteins, arrestins, signaling pathways and ion channels

Getting more specific, what leads to the psychedelic effects of psilocybin?

5-1. Blood psilocin correlates with experience. After ingesting the mushroom, psilocybin – which is actually a prodrug – is dephosphorylated in the stomach to psilocin – the active form – some of which crosses the blood-brain barrier and interacts with 5-hydroxytryptamine receptors. However, less than half of it makes it there due to the liver's "first pass" effect. In the liver, monoamine oxidase degrades about five percent, while another enzyme called UGT adds a sugar to about fifty percent. The trip ends after most of the psilocin is degraded to these non-hallucinogenic metabolites. As shown by the solid lines here, in a 2019 study, researchers found that blood levels of psilocin were dose dependent, and correlated well with the perceived intensity of the experience.[46] Both perceived intensity and blood psilocin level indicate that a psilocybin trip lasts roughly six hours. Scientists expect that a trip might be prolonged by inhibiting these enzymes, but this has not yet been studied.[47,48]

Blood concentration is one way of measuring the *magnitude* of overall experience, but how do scientists measure the different experiential qualities of psychedelics?

5-2. Different psychedelics have different impacts. Altered Consciousness States tests [49] are common ways to assess psychedelic experiences, with one version assessing 11 characteristics that represent three major qualities: visionary restructuralization, anxious ego-disintegration, and oceanic boundlessness. In this test,, psilocybin in red and LSD in blue both have mostly visionary restructuralization. MDMA in purple has mostly oceanic boundlessness and ketamine in green has mostly ego-disintegration.[50–52] This is consistent with psilocybin and LSD both acting through the same serotonin receptors.

So, which receptors are mainly involved?

5-3. 5-HT_{2A} Receptor plays key role in the psilocybin action. As seen here, both psilocin and LSD have sub-micromolar affinities for 6 serotonin receptors.[53] This means that they are capable of binding to different receptors, however that doesn't mean there will be equal effects through all of them. Conclusive evidence that the 2A receptor was the most important receptor for psychedelic effects came from a 1998 human study. Subjects were given psilocybin followed by fairly selective 5-HT_{2A} antagonists ketanserin or risperidone – or a different antagonist. As shown here for visual disturbances, the effects of psilocybin were only fully blocked by the 5-HT_{2A} antagonists in a dose-dependent manner.[54] Further work has supported this conclusion, including

a recent human study, showing how the binding to 5-HT_{2A} receptors increases with increasing psilocin blood levels.[46]

With the 5-HT_{2A} receptor identified as the important one, the question now becomes what is the difference between how hallucinogens like psilocybin and LSD interact with it, versus non-hallucinogens like ergotamine.

5-4. Hallucinogenic and Non-Hallucinogenic 5-HT_{2A} Receptor Agonists suggest biased agonism. Shown here is a binding curve demonstrating that the non-hallucinogenic compound ergotamine has a strong affinity for the 5-HT_{2A} receptor.[55] This reinforces that strength of binding alone is not a predictor of hallucinogenic potential. Recall that via biased agonism various molecules can create distinct downstream effects based on how they bind to a receptor. One informative study used mice in which 5-HT_{2A} receptors were removed to compare the effects of hallucinogens with non-hallucinogens in terms of what genes they turned on after they interact through the 5-HT_{2A} receptor. All the hallucinogens turned on both *egr-2* and *c-fos* genes, whereas non-hallucinogens only turned on the *c-fos gene*. This difference makes it clear that biased agonism plays a role in the effects of psilocybin and other classic psychedelics. The same research group further showed that by only expressing 5-HT_{2A} receptors in the cortical neurons of mice, it restored the apparent hallucinogenic activity, proving it is those neurons rather than any others that are responsible for the psychedelic effects.[56]

5-5. Take-home #5. This leads us to our fifth take home message which is, the psychedelic effects of psilocin require its action as a biased agonist of cortical neuron 5-HT_{2A} receptors with details presumably impacted by other receptors.

Now let's continue by very briefly considering functional impacts at the level of the brain.

6-1. Layer 5 pyramidal neurons. As was just noted, the hallucinogenic effects of psilocybin are due to its action in cortical neurons.[39] Interestingly, functional MRI studies that measure blood flow as a proxy for brain activity, show that psilocybin leads to decreased cortical activity – a center of information integration and management – and increased activity in the medial temporal lobe – a region active during dreaming and associated with memory formation and retrieval.[57] The cortex has layers, and layer 5 has large pyramidal neurons that are particularly rich in 5-HT_{2A} receptors.[58] These neurons make thousands of connections with other brain parts and project heavily onto inhibitory neurons, playing an inhibitory "pacemaker" function synchronizing brain rhythms.[51] Magnetoencephalography studies of volunteers show that psilocybin decreases the amplitudes of all brain waves … shown here as pink/light colors, with an especially dramatic impact on alpha-waves – waves that are associated with a resting brain. The explanation given is that Psilocybin excitation of the cortical layer 5 neurons causes them to fire irregularly, disrupting their pacemaker activity, desynchronizing the resting rhythms of the brain … so synchronization of brain waves decreases while overall activity increases.[59]

6-2. The "Brain entropy" hypothesis. Robin Carhart-Harris, head of the Imperial College London Center for Psychedelic Research, has captured these ideas in his "entropic brain" hypothesis. Brain entropy or randomness is marked by a decrease in activity in managerial hub regions and increased activity in other areas, causing an overall higher but dysregulated brain activity, including disrupting what is known as the "Default Mode Network". This striking colorful visual image of the increased randomness comes from analyzing functional MRI data using a computational network analysis to analyze connectivity between individual brain regions. It shows that psilocybin (on the right) leads to both many more and many novel low-strength connections and a weakening of some normally strong connections.[60] This image shows the idea of normal

consciousness as existing between a low-entropy unconsciousness that can be induced by sedatives and a high entropy unconsciousness that psychedelics push toward.[61,61–63]

That is all interesting, but how do the hallucinations come about?

6-3. What's behind the hallucinations? As was mentioned, the decrease in brain alpha waves caused by psilocybin increases the excitation level of cortical sensory networks. To assess how this might relate to the hallucinations induced by psilocybin, researchers measured the alpha waves in the visual parieto-occipital network during a recognition task. Data with a placebo show high levels of alpha waves level that decrease dramatically with a visual stimulus reflecting the stimulus-induced increase in activity. With psilocybin, the prestimulus alpha-waves are drastically reduced implying there is already a much higher level of neuronal excitement even without a visual stimulus.[64] Using ketanserin to block the 5-HT2A receptors and hallucinations, the alpha waves remain high, confirming that the alpha wave decrease is due to 5-HT2A receptor activation and its presence correlates with hallucinations.[65] Thus, the alpha wave decreases and the associated increased excitability of the visual network in the absence of visual inputs appear to be the source of the visual hallucinations.

6-4. Take-home #6. At a systems level, psilocybin appears to desynchronize inhibitory cortical brain activity exerting a "disorganizing" influence and expanding interactions.

A separate question is whether psilocybin has therapeutic potential? This is best answered by looking at clinical studies involving psilocybin.

7-1. Studies of psilocybin's long-term therapeutic effects. An excellent review of modern psilocybin clinical studies by Aday *et al.* compiled 34 experimental studies on the long-term effects of psychedelic drugs.[66] Psilocybin was used in 28 of the studies, and of these, 9 focused on depression. Looking at long-term effects is important because the efficacy of many current antidepressant treatments diminishes over time. It is therefore notable that in some studies, psilocybin had long-lasting positive effects on depressive symptoms.

7-2. Psilocybin for treatment of depression. Studies of mental health treatment with psilocybin done before the Controlled Substances Act are not comparable to modern studies. This is due to altered classifications of mental illness as well as the standardization of inventories used to quantify psychiatric symptoms, such as those associated with anxiety and depression. Here, we will just look at 2 modern studies investigating the efficacy of psilocybin for treating clinical depression. Both studies used standardized inventories to quantify depressive symptoms, with scores falling into categories of increasing severity, ranging from none to mild to moderate to severe depression.

The first study, from 2016, was a pilot study using psilocybin for treatment-resistant depression. Patients received two oral doses one week apart, with psychological support provided throughout each session, (Pause) and were assessed at intervals for up to 3 months after receiving the second dose of psilocybin. As you can see, the average QIDS score of the 12 subjects decreased significantly from severe to mild, with the effect still significant at 3 months post-treatment.[67] A larger 2021 study with a similar protocol showed promising results as well. These 24 study participants also received two doses, 1.6 weeks apart on average, and each session was conducted with supportive psychotherapy. Patients experienced a significant decrease in their depression score, and it remained low for a month after treatment. Both studies concluded that psilocybin has promise as an efficacious treatment for depression, since no serious adverse effects were reported and symptom improvements were maintained long-term.[68]

Results like these led the FDA in 2018 to designate psilocybin as a “breakthrough therapy” for treatment-resistant depression and in 2019 for major depressive disorder. This is only granted when evidence suggests that a drug may be an enormous improvement over available therapies, and is meant to accelerate progress through necessary clinical trials.

7-3. Ongoing psilocybin clinical trials. According to clinicaltrials.gov, there are currently 29 active studies involving psilocybin. The main conditions targeted by these trials are treatment resistant depression, major depressive disorder, and substance dependence. As can be seen here, no phase 3 or phase 4 studies are yet underway. So in terms of the formal drug approval processes, focus remains on establishing drug safety, determining adverse effects, and gathering preliminary data on effectiveness for the condition in question.[66] Despite ongoing struggles to obtain funding and the drug itself for research purposes, the FDA approving psilocybin as a “breakthrough therapy” should accelerate progress toward Phase 3 trials.

7-4. Psilocybin microdose impacts appear to be due to a placebo effect. One different mode of psychedelic use is taking sub-hallucinogenic doses. A remarkable cleverly designed study was published just this year: a large - 191 subject – double-blinded, citizen science study of microdosing psilocybin or LSD. The microdose amount was defined by each volunteer as the volunteers were not identified but were given envelopes with 4 capsules each week for 4 weeks. Volunteers emailed self-reported outcomes back to researchers, including their guess of whether they got a placebo or microdose. The placebo group got placebo all 4 weeks, the microdose group got 2 microdose and 2 placebo each week, and a half-and-half group got 2 microdose and 2 placebo in weeks 1 and 3 and just placebo in weeks 2 and 4. The results are striking. In this plot, subjects getting the placebo are in green and those getting the microdose in red, grouped by what they thought they got. As you can see the actual pill taken did not impact a subject’s emotional state, but if they thought they got the microdose, they had an improved emotional state. The same pattern of significant differences was seen for the other self-reported outcomes except cognition. The authors’ conclusion was: “The findings suggest that anecdotal benefits of microdosing can be explained by the placebo effect.”[69]

7-5. Take-home #7. So take-home#7 is that psilocybin/psilocin has therapeutic potential, but microdose impacts appear to be due to a placebo effect.

While clinical trials are a must for determining efficacy in people, animal models also play very important roles.

8-1. Mouse models are useful. So, what are ways to study psilocin responses and events in the brain considering we cannot directly measure what is going on in the brains of living people? Animal models are extremely valuable, allowing for studies which would be unethical in humans. Mouse models are particularly useful as they are mammals with shorter lifespans that are relatively low cost to work with. However, to be relevant to humans, mouse models must be rigorously validated, ensuring they share key characteristics with the disease or disorder being modeled and that similar mechanisms are involved. A valid animal model allows researchers to identify biological markers and tissue changes as well as test treatments ahead of human clinical trials.[70,71]

Diseases and disorders successfully modeled in mice include Alzheimer’s, cancers, PTSD, OCD, schizophrenia, anxiety, and relevant to today’s talk, depression. How exactly do researchers model a disorder such as PTSD or depression? It's not as if they can just ask mice if they are depressed or stressed. So, researchers must be inventive—For instance, to induce an important marker of depression, a lack of desire to pursue pleasure, researchers have used both forced

swim tests in which mice were put in water for 6 minutes, and a multimodal stress consisting of restraint, light, loud music, and jostling for hours at a time. Just as stressed people can become depressed and less interested in pleasurable experiences, researchers find that after these treatments mice are less interested in both tasty food and sex.[72]

8-2. Head-twitch response. To test impacts of psychedelics like psilocin, the need for a valid model is again quite clear as we cannot simply ask mice if they are hallucinating. A key discovery was made by Keller and Umbreit in 1956, about how mice respond to hallucinogens. This behavior is known as a head-twitch response, in which mice twitch their heads back and forth in a rhythmic pattern.[73] Another experiment displayed interesting data which showed that classic hallucinogens all cause the head twitch response, yet mice with their 5-HT_{2A} receptor knocked out do not, powerfully reinforcing that these molecules all act through the 5-HT_{2A} receptor.

This response is not only a very useful, simple indicator of whether a substance will cause hallucinations in humans, but also can assess the hallucination potency of a drug. As seen here, a study comparing 36 different psychedelics in mice, found that the potency of a hallucinogen in humans matches well the potency of the head-twitch response in mice.[74]

8-3. Take-home #8. Given this, take home 8 is that animal models are very useful for dissecting biochemical mechanisms and for preclinical exploration of potential therapeutic uses.

Another important area opened up by animal models is the study of neuroplasticity**.**

9-1. Psychoplastogens. You may be thinking, “what is neuroplasticity?” Neuroplasticity is the ability of neurons to make new connections and is a measure of the brain’s ability to adapt and repair. It is essential to learning and memory and potentially can offset the atrophy of cortical neurons thought to contribute to the development of mood and anxiety disorders. Remarkably, recent studies show that many psychedelics promote neuronal growth leading scientists to coin the term “psychoplastogen” meaning “mind-shape-generating”.[75,76] In 2018, a breakthrough study tested 6 hallucinogens, including psilocin, for neuroplastic activity on cultured *Drosophila* cortical neurons using antibody labeling to visualize the neurons. Cells treated with LSD, DOI, noribogaine, MDMA, psilocin, or DMT *all* showed visibly increased complexity, and quantifiable increases in crossings, dendritic branches and other parameters not shown. Zooming in, they also showed large increases in dendritic spine formation, with representative data shown here.[77]

9-2. Neuroplasticity appears to require Brain Derived Neurotropic factor. Taking this a step further, they looked into the mechanisms behind the neuroplasticity using DMT activation of cultured cortical pyramidal neurons. Using the 5HT2A receptor antagonist ketanserin, they showed that the growth promoting effects required the 5HT2A receptor. Noticing the effects were similar to those induced by the brain derived neurotropic factor (BDNF) they checked if the effect was independent of BDNF. Their simple approach was to treat neurons with the hallucinogen DOI or with BDNF, or both together. This revealed DOI and BDNF do not have an additive effect on neuroplasticity implying they do not act independently.[78] Since BDNF is known to signal through its receptor and the protein known as mTOR, they tested if these were required for the DMT-induced neuroplasticity. Indeed, a BDNF receptor antagonist or a known mTOR antagonist both fully extinguished the neuroplasticity.[77] Thus, these hallucinogens seem to promote neuroplasticity through increasing BDNF.

9-3. Take-home #9. Psilocin and other psychedelics promote neuroplasticity - potentially supporting long-term therapeutic effects.

One very interesting question that has not been addressed until recently is to what extent are the hallucinogenic effects required for potential therapeutic activities of psychedelics.

10-1. Refining a Psychedelic to Improve Clinical Effectiveness. In 2020, NIH funded researchers at UC Davis reported modifying a classic natural hallucinogen to eliminate the psychedelic effects while maintaining its neuroplastic activity and therapeutic effectiveness in an animal model.[79] The group took Ibogaine, from the plant *Tabernanthe iboga*, which has limited use in psychotherapy due to it being difficult to synthesize, its accumulation in adipose tissue which contributes to its cardiotoxicity, and its ability to cause 24 hour long hallucinations. They systematically eliminated different moieties until they found Tabernanthalog or TBG. This compound was easy to synthesize, and in mice had little to no cardiotoxicity and did not cause hallucinations. TBG also promoted neural plasticity as measured by increased dendrites in rat embryos, and in a forced swim test, rats treated with TBG were more active and seeking escape, similar to the effect of ketamine, indicating a less depressed state.

10-2. Tabernanthalog also maintains anti-addiction activity. In the same study, TBG was shown to reduce addictive tendencies in mice.[79] First, when administered during binge drinking of alcohol, one treatment with TBG resulted in significantly reduced ethanol intake. As a step further, heroin was tested, an extremely addictive opioid. For this trial mice could self-administer heroin by pressing a lever, which would also turn on a light pairing these two events. The behaviour was then extinguished by removing heroin and the light. Lastly the light was added back to see if lever pressing would restart indicating addiction. It was found that TBG acutely reduced heroin intake during the self-administration stage and blocked habit reformation if taken within 12 days of light reinstatement. Together these results imply that the antidepressant, anti-addictive, and neuroplastic properties of ibogaine are independent of its psychedelic effects, even though they also depend on the 5-HT_{2A} receptor.

So might some of psilocybin's potential therapeutic activities be independent of its psychedelic effects?

10-3. Antidepressant behavior is separable from the 5-HT_{2A} receptor. In a paper published this spring, the 5-HT_{2A} antagonist ketanserin was used to show that using a rodent model, psilocybin psychedelic effects were not essential for its antidepressant activity.[70] First, male rodents went through a pleasure training using 1% sucrose and urine from females in estrous, representing good food and sex, and then were tested to establish the baseline preferences. Rodents were then given multimodal stress for 10-14 days, followed by a second test. Then treatment with saline, the 5-HT_{2A} antagonist ketanserin, and/or psilocybin, followed by the last test. The stressed mice sought less pleasure, and psilocybin (in yellow) significantly restored their pleasure compared to control (in grey), while blockage of 5-HT_{2A} with ketanserin alone (in blue) had no effect … but surprisingly, it did not block the effect of psilocybin, implying that the antidepressant-like activity of psilocybin does not depend on the 5-HT_{2A} receptor.

10-4. Antidepressant behavior is separable from mice head twitch response. They also asked whether the antidepressant activity was separable from the head twitch response.[70] As expected, psilocybin treatment (in yellow) significantly increased the head twitching as compared to control and ketanserin and 5-HT_{2A} blockage by ketanserin lower the number of head twitches. However, as one mouse seems unresponsive to psilocybin and two in the ketanserin group did not lower head twitching, they reanalyzed the data in high and low head twitching groups. This led to an even higher significance (p value < 0.001). The authors concluded that "Our preclinical results therefore suggest that 5-HT_{2A}Rs, and thus psychedelic responses in humans, may not be

required for an antidepressant response to psilocybin, although that can only be definitively established with tests in humans." So, the take home for this section is

10-5. Take-home #10. Some therapeutic effects of psilocybin appear to be separable from the psychedelic effects

Sum-1. Summary/outlook. We hope you have found this information-rich tour of the science of psilocybin as fascinating as we have, and that it has piqued your interest about one thing or another. And while much is known about psilocybin, there is of course much more still to be learned. I am very proud of these students who, starting from scratch, dug through literally hundreds of research articles, to distill out what they shared today. They – and hopefully you also now – have a better sense of how deep and complex, but also how rich the scientific literature is in terms of what can be learned from it.

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