Psilocybin reduces depressive-like behavior and improves cognition in healthy aging mice via epigenetic regulation of plasticity- and immune-related genes

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Abstract

For many, cognitive and affective health declines through typical aging. Although cognitive and affective symptoms are often studied in isolation, they share substantial overlap, and arise, in part, from common biological processes. Aging is accompanied by diminished neural plasticity, heightened neuroinflammation, and widespread alterations in the epigenome. These molecular changes mirror behavioral decline, linking the erosion of cellular adaptability to the decline of cognitive function and emotional well-being in aging. Here, we show that psilocybin reverses age-related behavioral and epigenetic alterations in aged mice. Male and female C57BL/6 mice (11 months old) received two intraperitoneal doses of psilocybin (1mg/kg) or saline one week apart and were evaluated for memory and affective behaviors. Psilocybin improved learning and memory in females and reduced depressive-like behavior across sexes. Genome-wide DNA methylation profiling in the prefrontal cortex and bilateral hippocampus revealed widespread, sex- and region-specific effects, with the right hippocampus of females showing the most extensive gene-level changes. Differentially methylated loci were enriched for pathways related to synaptic organization, axon guidance, and neuroimmune signaling. Notably, psilocybin reversed age-associated methylation at CpG sites linked to typical aging, including within the *Tbr1* promoter, a transcription factor essential for excitatory neuron development and synapse formation. Moreover, methylation at *Tbr1* mediated psilocybin's pro-cognitive effects on Y-Maze performance in females. Together, these findings demonstrate that psilocybin induces coordinated behavioral and epigenetic remodeling in the aging brain, with lateralized and sex-dependent signatures implicating neuroimmune and neuroplasticity transcriptional networks. Psilocybin thus emerges as a candidate compound for promoting aging resilience.

Significance

Psilocybin enhanced cognition and affective behavior in aging mice while reversing age-associated DNA methylation signatures in the hippocampus. These effects were sex- and hemisphere-specific and largely converged on neuroplasticity and immune signaling pathways. The findings reveal epigenetic mechanisms through which psilocybin may promote neural resilience and counteract brain aging.

Introduction

As we age, roughly two-thirds of Americans experience cognitive problems[1], one-third suffer from mild cognitive impairment or dementia[2], one-fifth report mood symptoms[3], and around one in every ten is diagnosed with depression[4]. Though cognitive and mood symptoms are traditionally studied separately, they are highly comorbid[5–7], especially during aging[3], and both are partially driven by decreased plasticity and increased inflammation in the brain[8–15]. Changes in DNA methylation are considered a molecular hallmark of aging[16], with decreased expression of neuroplasticity genes and increased expression of inflammatory genes repeatedly linked to age-related cognitive decline and mood symptoms[8–10,12,17,18].

Psilocybin, a naturally occurring pro-drug to the psychedelic compound psilocin, has garnered significant attention for its clinical benefits. Psilocybin products are being developed for Major Depressive Disorder (MDD) and Treatment-Resistant Depression (TRD) following positive results from multiple multi-site phase 2 trials[19,20], and there is at least preliminary evidence of its efficacy in numerous other indications, including other psychiatric disorders[21–29], inflammatory diseases[30,31], and pain-related conditions[32]. Beyond these effects, growing evidence indicates that psilocybin also enhances cognition; improving creativity, cognitive flexibility, and working memory in both healthy individuals and clinical populations[33–36]. In the brain, psilocybin produces widespread pro-neuroplastic effects, including increased expression of neuroplasticity- and inflammatory-related genes[37,38], growth factor signaling[39], dendrite and spine density[40,41], temporal-ammonic synaptic strength[42], and hippocampal neurogenesis[41]. Some of these effects appear within 24 hours and persist for upwards of 30 days[40], reminiscent of the immediate and sustained symptom reduction seen in clinical trials with psilocybin.

Although psilocybin shows promise for improving mood, cognition, neuroplasticity, and neuroinflammation, outcomes that are key for healthy brain aging, its effects in aging remain largely unstudied. One recent study demonstrated that monthly administration of high-dose psilocybin to aged mice extends longevity[43], but whether psilocybin reduces age-related emergence of mood and cognitive detriments remains unknown. The prefrontal cortex (PFC) and hippocampus – regions critical for cognition, mood regulation, and synaptic plasticity – are particularly vulnerable to age-related decline[44–47]. Moreover, dysregulation in PFC–hippocampal circuitry contributes to both cognitive impairment and depressive phenotypes in aging[48–50].

Here, we tested whether psilocybin treatment forestalls the development of cognitive and mood symptoms through neuro-epigenetic mechanisms that preserve PFC–hippocampal plasticity and counteract age-related shifts in DNA methylation. Healthy 11-month-old male and female mice received two psilocybin or control treatments and then underwent a behavioral battery assessing learning, memory, anxiety-, and depressive-like behaviors, after which brain tissue from the PFC and hippocampus was collected for epigenetic analysis.

Methods

Animals

Thirty 11-month-old male and female C57BL/6 mice were ordered from The Jackson Laboratory[51] and housed in a temperature- and humidity-controlled facility on a 12-hour reverse light/dark cycle. Mice were group-housed (n=5) with other mice of the same sex and treatment condition and allowed *ad libitum* access to standard chow and water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Arizona State University and conducted in accordance with NIH guidelines for the care and use of laboratory animals. Mice were allowed to habituate for one week upon arrival before treatment initiation, and all procedures were conducted between 7:00am and 7:00pm, during the dark cycle.

Treatment

Animals received two intraperitoneal injections of either saline or psilocybin (1mg/kg; 0.35mg/ml) one week apart (**Figure 1**). Co-housed mice were injected simultaneously and returned to their home cage immediately after injection. Behavior testing began three days after the second injection.

Behavior

Learning and memory were evaluated with a Y-Maze[52–54] adapted for water escape to facilitate task acquisition and prevent satiety-related demotivation. On the first day of testing, animals received three habituation trials with no platform available for escape. Animals were then given five trials per day across three additional days to learn the location of an escape platform located ~3cm beneath the surface of the water, in either the left or right arm of the Y-Maze (each arm: 32 x 7 x 20cm). The platform location was counterbalanced across sex and treatment group and remained constant for each mouse across days and trials. Animals were given a maximum trial time of 60s, followed by 15s on the platform, and a 30s inter-trial-interval (ITI). Water was maintained at 18-20°C and colored with non-toxic white paint to obscure the platform location. Behavioral metrics included Errors, Latency, and Distance to the platform.

Memory was also evaluated with the Novelty and Location Recognition Tasks[55]. For Novelty Recognition, animals were given two trials across two days, during which two objects were placed in the corners of a square plexiglass arena (40 x 40 x 30cm), opposite from the drop-off point. For the first trial of each day, two identical novel objects were placed in the arena and animals were allowed to freely explore for 5m. On the second trial of each day, one of the objects was replaced with a novel object of similar dimensions and animals were given another 5m to explore. Animals were given a 30s ITI on the first day of testing, and a 45m ITI on the second day. Location of the novel object (left vs right) was counterbalanced across Sex and Treatment, and Time spent exploring each object was scored.

Location recognition methods paralleled Novelty Recognition, except on the second trial of each day, animals were re-presented with the same set of objects from the first trial with one moved to a novel location. Location of the moved object (left vs. right) was again counterbalanced across Sex and Treatment, and behavior metrics were identical to those used for Novelty Recognition.

A Novelty-Suppressed Feeding task was used to evaluate depressive-like behaviors. Mice were food restricted for 24h with continued access to water prior to testing, then placed in a novel environment made of black Plexiglas (40 x 40 x 30cm; filled with Sani-Chip bedding), with one pellet of standard rodent chow in the center of the arena. Each mouse was allowed to explore the arena freely for 5m to assess Latency to Feed, defined as initiation of chewing, holding, or placing the food pellet in the mouth.

Mice were evaluated in an Open Field to assess locomotion and anxiety-like behavior. Each mouse was individually placed in a square arena made of black Plexiglas (40 x 40 x 30cm; cleaned with 70% ethanol between trials to eliminate olfactory cues) and allowed to explore freely for 10m in the dark (under infrared light). Outcome measures included Distance traveled, number of Center Entries (the inner 33% of the arena), and Center Time.

The Marble Burying test was also used to evaluate repetitive and anxiety-related behaviors. Mice were placed in standard polycarbonate cages (20 x 30 x 13cm), filled with 5cm of clean, dry Sani-Chip bedding, and twenty glass marbles (1.5cm diameter), evenly spaced in a 4 x 5 grid on the surface of the bedding. Each mouse was allowed to explore the test cage for 10m, after which the number of Marbles Buried (defined as at least two-thirds covered by bedding) was recorded by an experimenter blinded to group assignments. The bedding was leveled between trials, and marbles were cleaned with 70% ethanol to remove olfactory cues.

DNA Methylation

Mice were deeply anesthetized using isoflurane (3-5%) until a surgical plane of anesthesia was confirmed by lack of reflexive response to toe pinch. Animals were then rapidly decapitated, and brains were immediately extracted and placed on an ice-cold dissection platform. Using surgical tools under a stereomicroscope, the ventromedial prefrontal cortex (PFC) was isolated by making coronal cuts approximately +2.0 to +1.5mm anterior to bregma and dissecting the medial portion of the prefrontal cortex ventral to the forceps minor. To isolate the left and right dorsal hippocampi, the brain was blocked by making coronal cuts at the anterior and posterior boundaries of the dorsal hippocampus (approximately −1.0mm to −3.5mm relative to bregma). The dorsal portion of the hippocampus was then dissected from each hemisphere. Dissected tissue samples were immediately placed in individual microcentrifuge tubes, flash-frozen on dry ice, and kept frozen at −20°C until DNA isolation.

The Applied Biosystems MagMAX DNA Multi-Sample Ultra 2.0 Kit was utilized to isolate DNA from frozen samples of brain tissue. Each sample was directly transferred into a well within a 96-deep-well plate, then treated with Enhancer Solution (20 ul), followed by Proteinase K(40 ul), and Extraction Buffer (250 ul) (Thermo Fisher Scientific). Plates were then sealed with adhesive film and incubated in a hybridization oven at 65ºC for 2 hours to facilitate enzymatic digestion and tissue lysis.

Following incubation, the plate was centrifuged at 1,500 RPM for one minute to encourage residual tissue debris to form a pellet. A maximum of 500ul of supernatant (lysate) from each well was transferred into a new 96-deep-well plate to avoid protein or particulate contamination. Each sample was then treated with 10ul of RNase A (not included in the kit; maintained on ice) and the contents of each well were thoroughly mixed and allowed to rest for ten minutes. Each sample was then treated with 400ul Binding Solution and Magnetic 40ul of Binding Beads (Thermo Fisher Scientific).

DNA purification was performed using the APEX Kingfisher instrument using a customized protocol developed specifically for 500ul of sample lysate. Following the isolation protocol, the sample yield and purity was quantified with a NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, United States) and normalized to 30ng/µl in 30µl.

The samples were bisulfite converted using Zymo EZ DNA Methylation- Gold™ Kit and hybridized to the Illumina Infinium Mouse Methylation BeadChip array[56] quantified on an Illumina iScanSystem. This array provides coverage of >285K markers across the mouse methylome for high-resolution epigenetic

analyses of murine strains. Raw Intensity Data (IDAT) files were preprocessed utilizing the Minfi package in R (for more details[57]). Probes known to be cross-reactive, located on sex chromosomes, and near SNPs were removed. Quality control analyses included quantile normalization, checking for sex mismatches, and excluding low-intensity samples (p<0.01)[58]. Methylation values were normalized and the R package TOAST[59] was used to generate estimated cell proportions.

Statistical Analysis

Behaviors were video recorded at 30 frames per second. For Y-Maze, Object Recognition, and Open Field tasks, body part tracking was done using DeepLabCut[60,61] (version 2.3.11). A custom MatLab script scored performance metrics in each task using nose-point coordinates. Novelty-Suppressed Feeding videos were hand-scored. Missing data, including corrupted or missing video files and un-trackable files (e.g., due to experimenter needing to lean over the maze to assist a mouse), were imputed using the R package MICE[62]. Novelty-Suppressed Feeding data were analyzed using Kaplan–Meier survival analysis and effects of Treatment, Sex, and their interaction were assessed using the log-rank test via the *survfit*() and *survdiff*()functions from the *survival* package in R (version 2024.09.0). Mixed models were then constructed in SPSS (version 29) for the Y-Maze, Open Field, Object Recognition, and Marble Burying tasks with fixed effects of Sex, Treatment, and a Sex x Treatment interaction, plus Trials nested within Days as a repeated measure for Y-Maze.

To evaluate the effects of psilocybin on DNA methylation, we performed an analysis of covariance (ANCOVA) at the CpG-site level, across all probes. For treatment predicting DNA methylation, our model included the following parameters: Treatment, Sex, Treatment x Sex, and all six Cell Count variables generated by the TOAST package. Then, gene-level significance was determined using an inverse variance weighting (IVW) approach applied across all CpG sites, followed by FDR correction at the gene level. Gene lists were generated separately for each contrast, excluding any genes showing significant Treatment x Sex interactions from the primary Treatment effect list. Each resulting gene list was analyzed using **STRING** to identify significantly enriched functional networks. Finally, genes showing significant main Treatment effects or Treatment × Sex interactions were combined for an overall Gene Ontology (GO) enrichment analysis using the enrichGO() function in the *clusterProfiler* package.

Aging-associated CpG sites were selected from published mouse epigenetic clocks, limited to probes overlapping with the Illumina Infinium Mouse Methylation BeadChip array[63,64]. A mixed model with fixed effects for Treatment and Sex, nine CpG Sites nested within the three Regions as a repeated measure, and all interaction terms, was used to identify aging-related epigenetic effects of psilocybin. Significantly affected aging-related CpG sites were then evaluated as potential mediators of psilocybin's behavioral effects (e.g., on YMaze in females and NSF in both sexes) using the Hayes PROCESS macro in SPSS. **Supplemental Table 1** provides a full list of age-related CpG sites that were evaluated.

Results

Effects of Psilocybin on Behavior

Psilocybin improved Y-Maze performance in females on Days 2-3 of testing, with the largest effects following overnight intervals [*Treatment x Sex x Day interaction for Errors:* $F_{(6,420)}$=3.02, p=0.007; *Treatment x Sex x Day x Trial interactions for Latency:* $F_{(32,420)}$=1.57, p=0.03, and *Distance*: $F_{(32,420)}$=3.32, p<0.001; **Figure 2a**). Male mice treated with psilocybin swam a longer Distance on the first two trials of Day 1 but otherwise performed at the same level as saline-treated mice. Thus, psilocybin enhanced spatial working memory in females, particularly after consolidation periods, and transiently increased exploratory activity in males, albeit without improvements in task accuracy.

Psilocybin treatment also affected Location Recognition performance on the first day of testing, with no delay between trials [*Treatment x Sex x Day x Trial x Object interaction:* $F_{(4,210)}$=4.14, p=0.003; **Figure 2a**]. On the test trial, psilocybin-treated female mice spent more Time with the *un-moved* object (p=0.001), suggesting a reduced preference for novelty, and psilocybin-treated male mice spent more Time with the *moved* object (p=0.015), consistent with enhanced novelty preference. Saline-treated mice did not show an object preference (*females:* p=0.32; *males:* p=0.68), signifying a lack of spatial recognition memory. Psilocybin treatment did not impact Novelty Recognition in males or females, either with no delay or with a 45m delay between trials [*Treatment x Sex x Day x Trial x Object interaction:* $F_{(4,210)}$=0.73, p=0.57, NS; **Figure 2a**], suggesting that psilocybin's effects may be limited to spatial forms of memory.

Psilocybin-treated mice ate sooner compared to saline-treated mice in the Novelty-Suppressed Feeding task [*median survival:* psilocybin=97.5s; saline=188s; $X^2_{(1)}$=4.10, p=0.04; **Figure 2b**], demonstrating that psilocybin treatment altered threat and/or reward appraisal in both sexes. Psilocybin treatment did not impact Center Time [$F_{(1,30)}$=0.38, p=0.54, NS] or Center Entries [$F_{(1,30)}$=0.19, p=0.67, NS] in the Open Field, or Marbles Buried in the Marble Burying task [$F_{(1,30)}$=0.12, p=0.73, NS]; however, there was a Sex-by-Treatment interaction for distance traveled in the Open Field [$F_{(1,30)}$=5.46, p=0.03; **Figure 2c**] where psilocybin increased locomotion in aging females (p=0.02), possibly representing enhanced exploratory drive, reduced behavioral inhibition, or mild arousal/motivational effects..

Effects of Psilocybin on DNA Methylation

Treatment with psilocybin altered DNA methylation at thousands of CpG sites on hundreds of genes in the PFC and hippocampus, and many of psilocybin's epigenetic effects showed sex differences and/or lateralization in the hippocampus (**Table 1**).

Psilocybin's effects on DNA methylation were highly region-dependent, with the largest number of effects in the right hippocampus (**Figure 3**), suggesting that psilocybin may preferentially modulate molecular pathways involved in memory formation, emotional processing, and stress regulation – key functions supported by the hippocampus.

Psilocybin's epigenetic effects were also highly sex-dependent, with females showing more alterations in DNA methylation overall following psilocybin treatment (**Figure 4**). This pattern may reflect sex differences in neuroplasticity, hormonal regulation, or stress responsivity, indicating that the molecular mechanisms underlying psilocybin's effects on the brain could differ substantially between males and females.

We found that functional enrichment networks related to nervous system development and neural architecture were the most affected by psilocybin across all brain regions in both males and females (**Figure 5a**), with additional sex-specific effects on networks related to immune response, organization neural circuits, and oligodendrocyte differentiation (**Figure 5b**). These patterns suggest that psilocybin broadly influences pathways supporting structural and functional plasticity, while engaging sex-dependent mechanisms that may differentially modulate neuroimmune signaling and steer circuit remodeling.

In the PFC, the functional networks most affected by psilocybin in both sexes were related to cell adhesion, with networks related to signaling and developmental regulation also heavily affected in males, supporting broad effects of psilocybin on neural communication and plasticity within the PFC (**Figure 6a**). In the left hippocampus, the top psilocybin-affected networks involved synaptic and cell projection structures, highlighting additional effects of psilocybin on neuronal connectivity and synaptic transmission in both sexes (**Figure 6b**). Psilocybin-altered networks in the right hippocampus were largely limited to females and enriched for cellular structures and processes central to synaptic connectivity and neuronal communication, indicating sex-specific modulation of genes involved in neurite outgrowth, axonal guidance, and the formation and maintenance of synaptic contacts (**Figure 6c**). **Supplemental Table 2** gives a full list of the functional enrichment networks that were affected by psilocybin within each brain region and sex.

Finally, analysis of known aging related genes (i.e., those from published 'epigenetic clocks') revealed Treatment effects on four out of nine tested, only in the right hippocampus of females [*Treatment x Sex x Region x CpG Site interaction*: $F_{(64,780)}$=31.01, p<0.001; **Figure 7**].

Importantly, psilocybin affected methylation at each CpG site in the *opposite* direction of typical aging, suggesting that psilocybin treatment may be capable of reversing the typical epigenetic aging trajectory. Moreover, methylation at cg0768739, located in the promoter region of the *Tbr1* gene, mediated the effect of psilocybin on YMaze Distance for the first trial of the second day of testing, where we found the strongest group differences in task performance in females, but not males [*Model Summary*: $F_{(3,26)}$=11.66, p=0.0001, R^2=0.57, Mean Square Error=0.0002; *Direct Effect*=163.10; SE=1304.45, 95% CI=(-2513.40 – 2839.61), p=0.90 NS; *Indirect Effect in Females*= -3246.16; SE=1487.75; 95% CI=(-6558.01– -811.96); *Indirect Effect in Males*=197.32; SE=457.19; 95% CI= (-549.03 1272.07), NS], implicating the T-box family of transcription factors in psilocybin's sex-specific pro-cognitive effects.

Discussion

This study is the first to demonstrate both cognitive and mood-related effects of psilocybin in an aging population, as well as the first to examine its impact on DNA methylation in brain tissue. Our findings provide compelling evidence that psilocybin engages molecular and behavioral pathways relevant to cognition and affective regulation in aged mice, and that many of these effects are, at least partially, sex-dependent. These results extend prior work in younger adult rodents and humans[19–23,40–42,65,66], and very old mice[43], highlighting psilocybin's potential as a therapeutic intervention to support cognitive and emotional resilience across the lifespan.

Consistent with clinical reports of psilocybin's antidepressant effects, we observed that psilocybin treatment reduced latency to feed in the Novelty-Suppressed Feeding task across both sexes, suggesting improved appraisal of threat and reward cues. This effect did not differ by sex and aligns with extensive prior work demonstrating psilocybin's rapid anxiolytic and anti-depressive properties in both preclinical and clinical studies[19–23,40–42,65,66]. Importantly, we observed no adverse effects on anxiety-like behavior in the Open Field or compulsive-like behavior in the Marble Burying task, indicating that acute psilocybin treatment does not produce deleterious behavioral outcomes in aged mice, which supports its safety profile in this context.

Beyond mood-related behaviors, we observed notable cognitive benefits in aging females, particularly in spatial working memory. Female mice treated with psilocybin exhibited improved Y-Maze performance, especially following overnight consolidation periods, suggesting enhanced memory retention. In the Location Recognition task, psilocybin modulated novelty preference in a sex-specific manner, with females displaying reduced preference for novelty and males showing enhanced preference, suggesting that psilocybin influences spatial recognition memory differently in males and females. This sex-specificity is supported by recent work implicating females as being more acutely responsive to psilocybin than males, both behaviorally (e.g., the Head Twitch Response) and biologically (e.g., Hypothalamic-Pituitary-Adrenal responsivity)[67], as well as evidence that the anti-addictive effects of psilocybin may be stronger in females[68]. However, published studies on the pro-neuroplastic and anti-inflammatory effects of psilocybin are mostly limited to males; one study that included female mice shows larger pro-neuroplastic effects of psilocybin in females[40]. Together, these findings suggest that psilocybin may sex-dependently support cognitive resilience during aging, while broadly enhancing motivational and reward-related behaviors in both sexes. This is a particularly important area for study, considering that women are twice as likely as men to develop dementias, such as Alzheimer's Disease (AD)[69].

At the molecular level, psilocybin induced widespread alterations in DNA methylation across the PFC and hippocampus. Importantly, these effects were often both brain region- and sex-specific, with the right hippocampus exhibiting the largest number of genes affected, particularly in females. This lateralized pattern in the hippocampus is consistent with fMRI studies showing that psilocybin and other classic psychedelics exert stronger impacts on right frontal and temporal regions; this has been reported for changes in relative perfusion[70], neurometabolism[71], and functional activation patterns[72]. In the PFC, functional networks most affected in males and females involved cell adhesion, signaling, and developmental regulation, supporting a role for psilocybin in facilitating cortical communication and plasticity. The effects observed in the right hippocampus, particularly in females, suggest that psilocybin may also uniquely and sex-dependently modulate epigenetic programs that underlie structural and synaptic hippocampal plasticity during aging – domains crucial for spatial learning and memory.

Results from a targeted analysis of aging-related CpG sites further highlighted psilocybin's sex-specific molecular effects. Psilocybin reversed age-associated methylation patterns in the right hippocampus of females at nearly half of the aging-related CpG sites examined, with one site in the promoter region of *Tbr1* mediating the sex-specific improvement in Y-Maze performance that we identified. *Tbr1* is a T-box transcription factor critical for neuronal differentiation and synaptic development[73–75], implicating canonical neurodevelopmental pathways in psilocybin's pro-cognitive effects. These results provide direct evidence that epigenetic modulation of plasticity-related genes contributes to the behavioral outcomes of psilocybin in aging females, offering a mechanistic link between molecular and cognitive effects.

Functional enrichment analyses revealed that psilocybin consistently targeted networks involved in synaptic structure, neuronal connectivity, and overall neuroplasticity across brain regions and sexes, with the right hippocampus in females showing particularly pronounced effects, consistent with the sex-specific effects on spatial learning and memory we observed. Key enriched categories included synapse organization, axon guidance, cell projection, and enzyme-linked receptor signaling, reflecting coordinated regulation of pathways that support synaptic transmission and circuit remodeling. These molecular signatures align with prior findings of psilocybin-induced dendritic spine growth and persistance[40], enhanced synaptic strength[42], and neurogenesis[41], suggesting that the presently observed DNA methylation changes may underpin psilocybin's known effects on structural and functional neuroplasticity.

Psilocybin also affected networks related to immune and inflammatory signaling, also in a sex-specific manner. These findings support accumulating evidence that psilocybin modulates neuroimmune pathways[34,76,77], which are increasingly recognized as critical contributors to age-related cognitive decline and mood disturbances[78–80]. By simultaneously engaging neuroplasticity- and inflammation-related networks, psilocybin may invoke dual mechanisms for enhancing neural resilience and mitigating deleterious effects of typical aging; an important implication for neurodegenerative disorders, such as Alzheimer's Disease.

Behavior-related enrichment networks further corroborated our sex-specific behavioral findings. In females, right-hippocampal networks were enriched for genes implicated in aggression, anxiety, fear learning, and consummatory behaviors, paralleling observed increases in exploratory drive and motivational behaviors. These results suggest that psilocybin modulates genes underlying emotional and cognitive processing in a complex manner that supports adaptive behavioral responses in aging females. The alignment between functional network enrichment, DNA methylation changes, and behavioral outcomes strengthens our interpretation that psilocybin engages integrated molecular programs supporting both cognitive and affective plasticity. Moreover, we find that psilocybin alters other behavior-related networks that have high relevance to its ongoing clinical development in substance abuse[24–27], such as hippocampal networks for nicotine and opioid addiction.

Our findings have several important implications. First, they demonstrate that psilocybin can enhance cognitive performance in aged females, extending preclinical and clinical evidence of psilocybin's pro-cognitive and antidepressant effects to an older population. Second, our sex- and region-specific epigenetic effects highlight the importance of considering biological sex and brain region when investigating psychoplastogens. Finally, the mediation of behavioral outcomes by DNA methylation at aging-related genes, such as *Tbr1*, provides a mechanistic bridge linking molecular and behavioral plasticity, suggesting potential targets for intervention.

Future work should explore whether repeated or chronic psilocybin administration produces cumulative or longer-lasting behavioral benefits, and whether these effects generalize to even older populations, as our is considered middle-aged for mice. Investigating the temporal dynamics of methylation changes relative to behavioral improvements will also be critical for understanding the causal pathways of psilocybin's effects. Furthermore, given the robust female-specific effects observed here, future studies should examine the interplay between sex hormones, aging, and epigenetic plasticity to identify mechanisms driving vulnerability and resilience.

In summary, this study provides the first evidence that psilocybin can improve cognitive function and modulate mood-related behaviors in aging mice, accompanied by widespread, sex- and region-specific DNA methylation changes. These epigenetic modifications converge on neuroplasticity- and immune-related networks, suggesting dual molecular mechanisms underlying psilocybin's effects. Notably, female-specific modulation of right-hippocampal DNA methylation, including at *Tbr1*, partially mediated cognitive enhancement, offering insight into how psilocybin may counteract age-related decline. Together, these findings position psilocybin as a promising candidate for promoting neural resilience and cognitive-emotional health in aging, with important implications for understanding sex-specific responses and guiding future translational psychedelic research.

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Table

Table 1 is available in the Supplementary Files section.

Figures

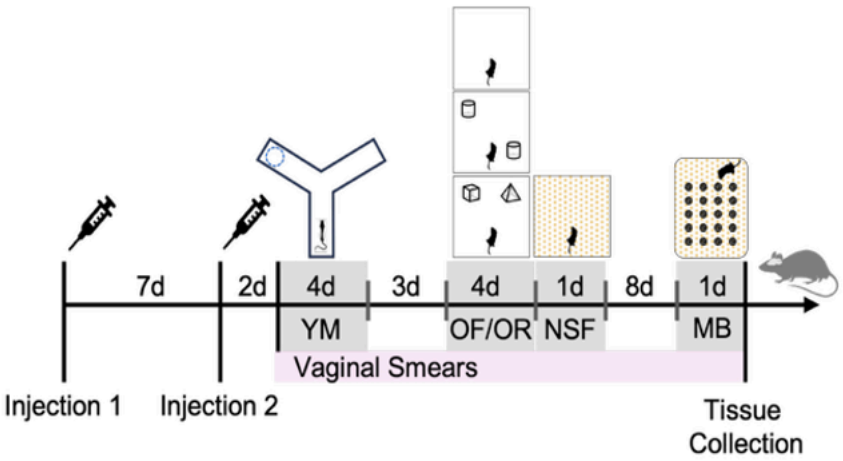

Figure 1

Overview of study design. Timeline of treatment administration, behavior tasks, and tissue collection. YM=YMaze, OF=Open Field, OR=Object Recognition, NSF=Novelty-Suppressed Feeding, MB=Marble Burying.

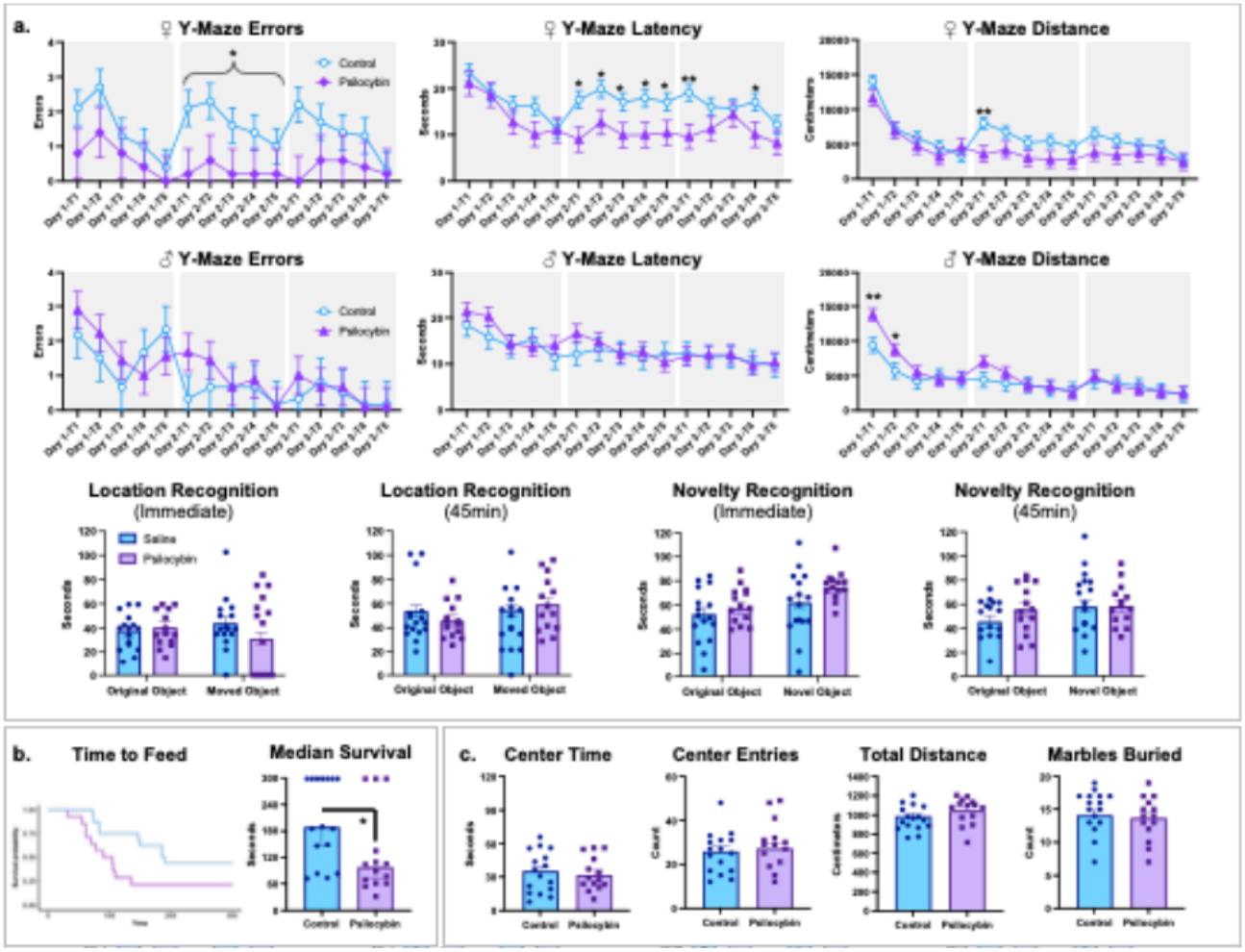

Figure 2

Behavioral effects of psilocybin. a) Cognitive tasks: Mean ± standard error for Y-Maze Errors, Latency, and Distance by Day, Trial, and Sex, and Time spent with each object on the recognition trial for Object Recognition tasks; **b) Depressive-like behavior:** Mean ± standard error with individual scores for Latency to Feed, and percent of each treatment group that had not yet eaten the pellet by the end of each 30-sec time bin for Novelty-Suppressed Feeding; **c) Anxiety-like behavior:** Mean ±standard error with individual scores for Center Time, Center Entries, and Distance in the Open Field, and Marbles Buried. *p<0.05, **p<0.01, T1=Trial 1, T2=Trial 2, T3=Trial 3, T4=Trial 4, T5=Trial 5.

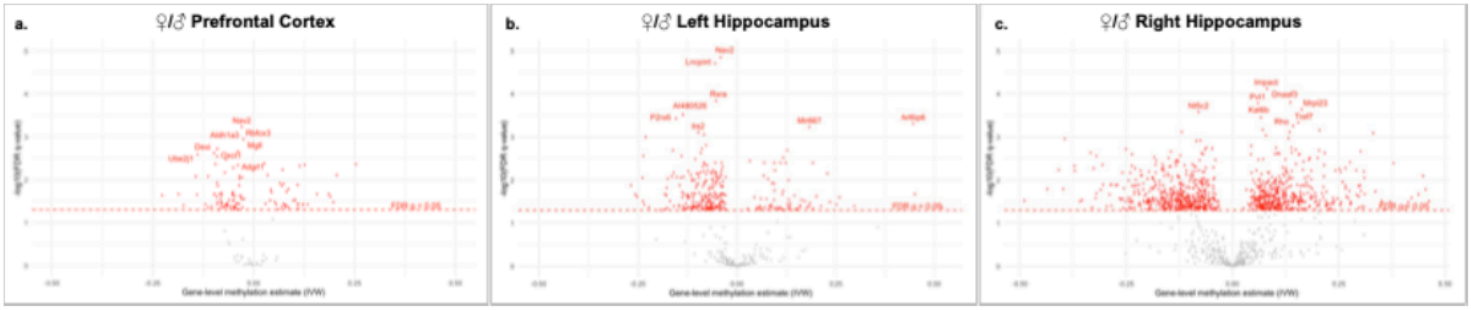

Figure 3

Sex-independent effects of psilocybin on DNA methylation. Volcano plots showing gene-level methylation estimates derived from inverse-variance weighting (IVW) in the: **a) Prefrontal Cortex**; **b) Left Hippocampus**; and **c) Right Hippocampus**. The x-axes represent the IVW methylation effect estimate for each gene, and the y-axes show the $-\log_{10}$ FDR-adjusted q-value. Each point corresponds to a gene-level methylation association, with points to the right indicating higher methylation estimates and those to the left indicating lower estimates. Genes passing the significance threshold (FDR q< 0.05) are depicted in red, reflecting loci with the strongest evidence of differential methylation.

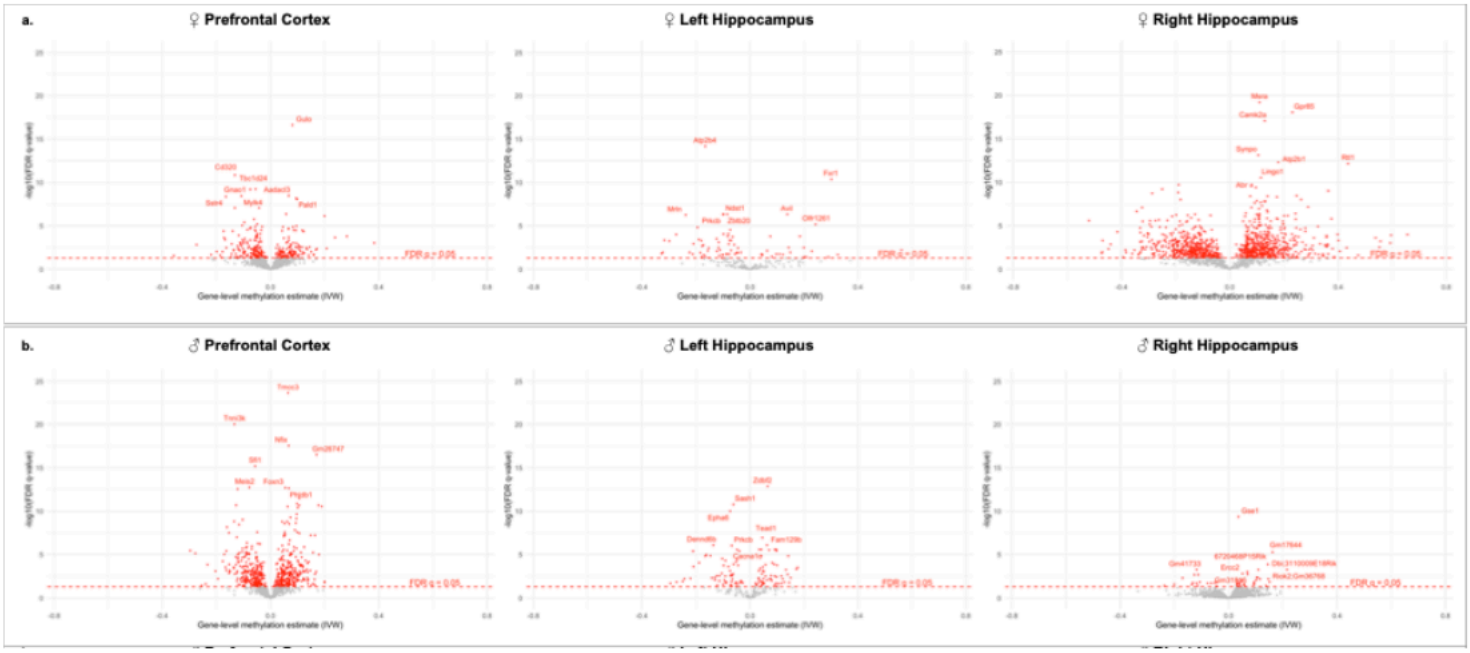

Figure 4

Sex-dependent effects of psilocybin on DNA methylation. Volcano plots showing gene-level methylation estimates derived from inverse-variance weighting (IVW) in the Prefrontal Cortex, Left Hippocampus, and Right Hippocampus of**: a) Females**; and **b) Males**. The x-axes represent the IVW methylation effect estimate for each gene, and the y-axes show the $-\log_{10}$ FDR-adjusted q-value. Each point corresponds to a gene-level methylation association, with points to the right indicating higher methylation estimates and those to the left indicating lower estimates. Genes passing the significance threshold (FDR q< 0.05) are highlighted, reflecting loci with the strongest evidence of differential methylation.

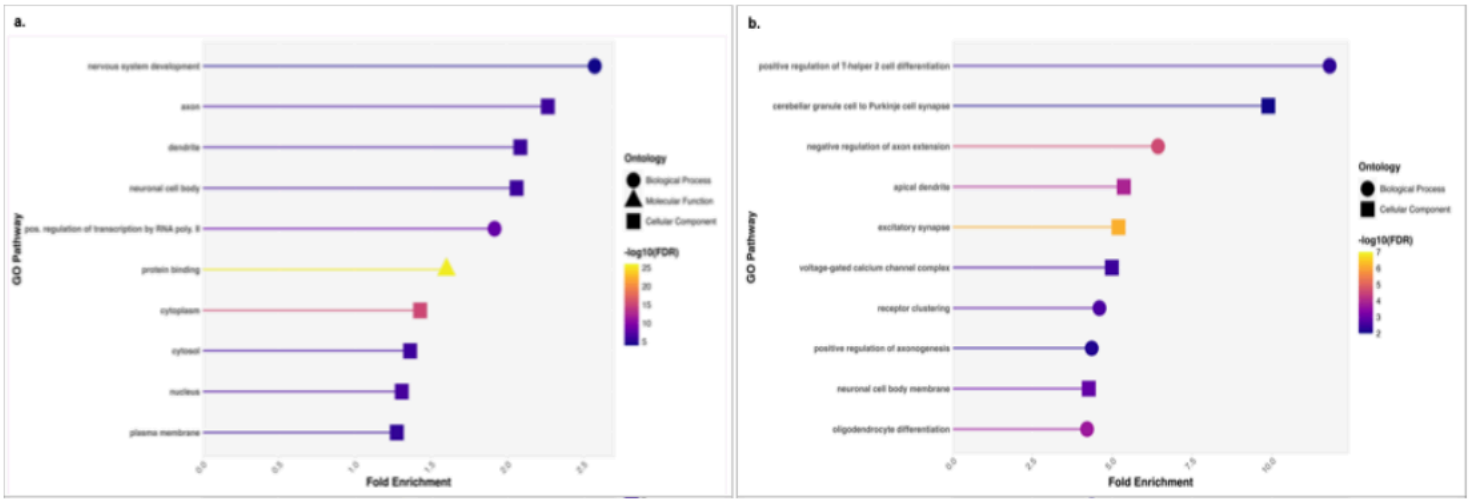

Figure 5

Top Functional Enrichment Networks affected by Psilocybin. Gene Ontology (GO) enrichment analysis of significant gene hits across all brain regions for **a)** Treatment; and **b)** Treatment by Sex interaction. The top 10 enriched pathways with the largest fold enrichment that met FDR<0.05 are shown. Pathways are colored by statistical significance (FDR) and shaped by ontology category (Biological Process, Molecular Function, Cellular Component).

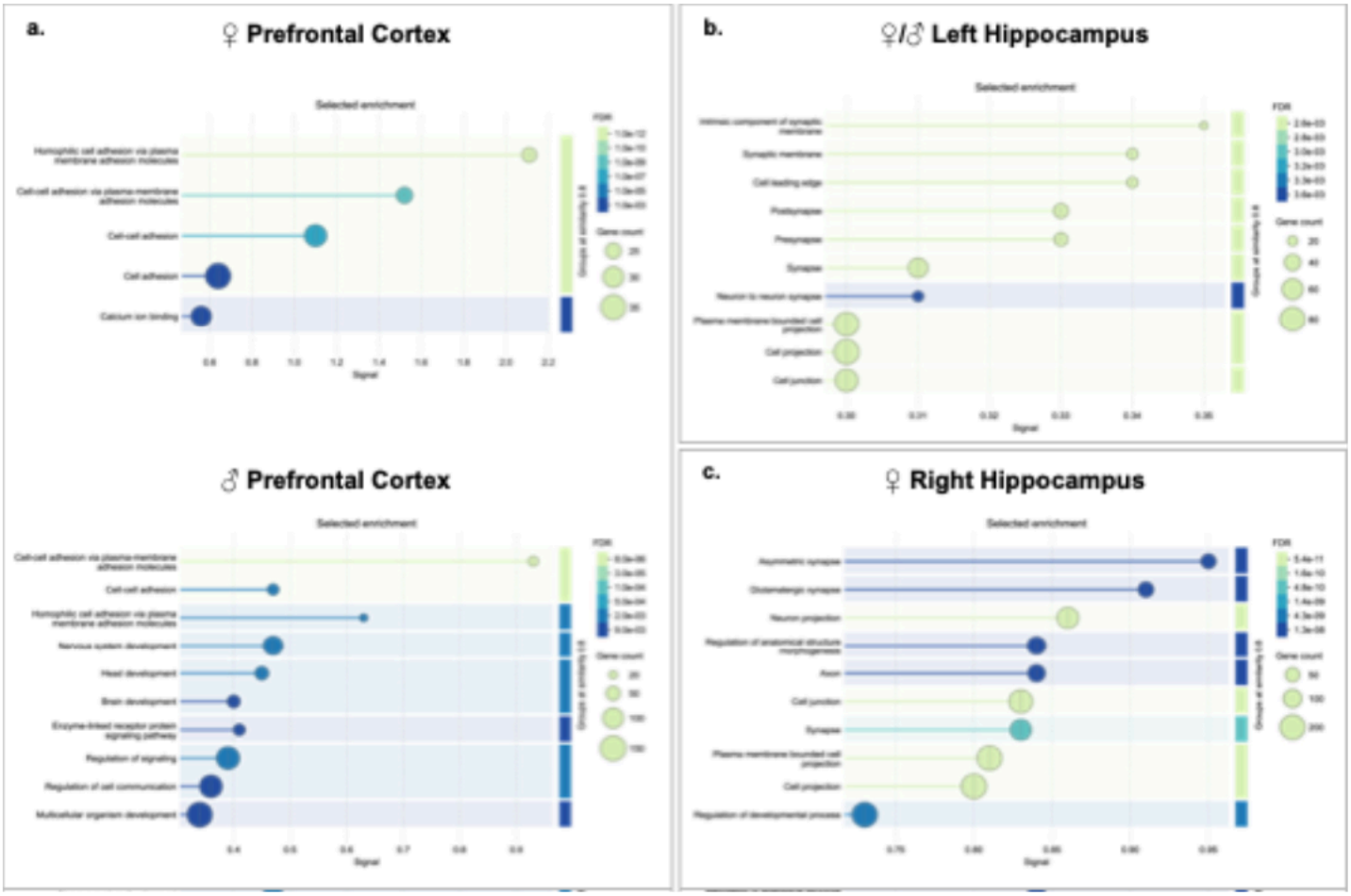

Figure 6

Top Functional Enrichment Networks affected in each Brain Region. Functional enrichment analysis of significant gene hits across all brain regions for **a)** PFC; **b)** Left Hippocampus; and **c)**Right Hippocampus. The top ≤10 GO enriched pathways with the largest signal that met FDR<0.05 are shown, grouped by ontology category (Biological Process, Molecular Function, Cellular Component). Pathways are colored by statistical significance (FDR) and shape sizes represent the number of genes that contribute to each network.

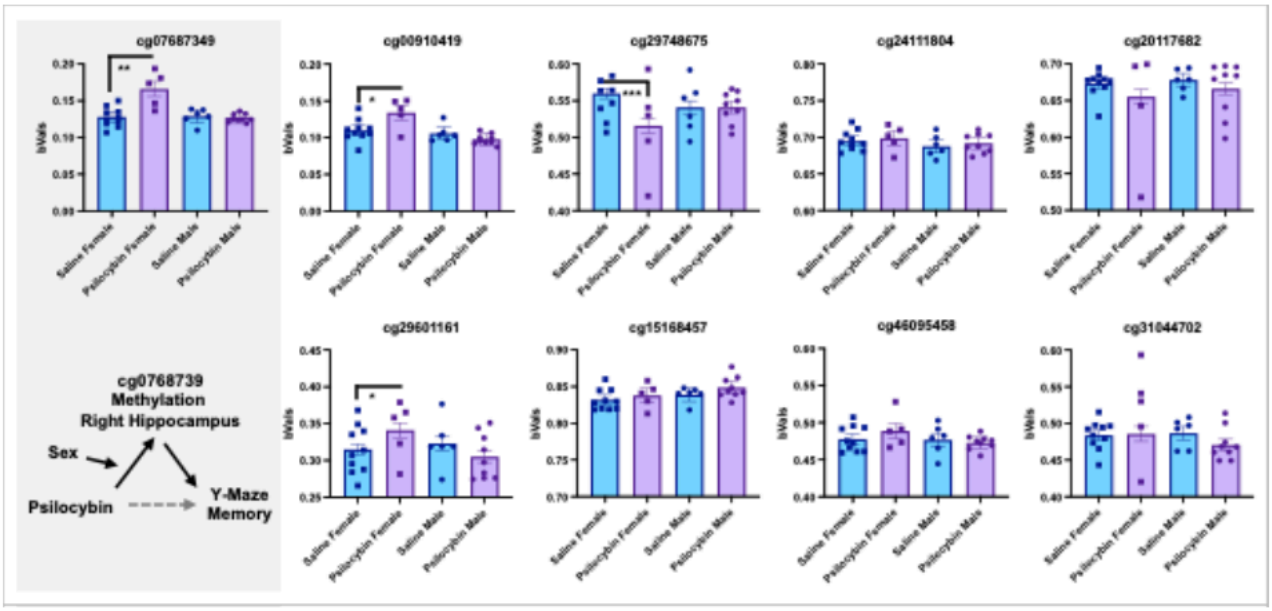

Figure 7

Effects of Psilocybin on Epigenetic Aging. Means + Standard Errors and individual scores for each Treatment and Sex in the right hippocampus, and model showing the sex-moderated mediation of psilocybin on YMaze performance.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PsiloAgingSupplementalTable2a.csv
- PsiloAgingSupplementalTable1.csv
- PsiloAgingSupplementalTable2d.csv
- PsiloAgingSupplementalTable2c.csv
- PsiloAgingSupplementalTable2b.csv
- PsiloAgingSupplementalTable2e.csv
- Table1.docx